ABSTRACT BOOK

FEMS 2019

8th Congress of European Microbiologists

In collaboration with sfam society for applied microbiology

7-11 July 2019 | Glasgow, Scotland | www.fems2019.org

TABLE OF CONTENT

O001 Systems metabolic engineering of microorganisms for the production of chemicals and materials from renewable resources

Sang Yup Lee[1]

[1]*KAIST, Chemical and Biomolecular Engineering, Daejeon, Korea, Rep. of South*

Background: Bio-based production of chemicals and materials from renewable non-food biomass is becoming increasingly important.

Objectives: To efficiently construct microbial cell factories, it is important to perform systems metabolic engineering that integrates traditional metabolic engineering with systems biology, synthetic biology, and evolutionary engineering.

Methods: Systems metabolic engineering startegies were developed and employed for the development of microbial strains capable of efficiently producing various chemicals and materials.

Results: Microbial strains developed for the production of dicarboxylic acids, diamines, aromatic compounds, polymers, and natural compounds will be presented with focuses given on systems metabolic engineering strategies employed.

O002 The bacterial pathogen Listeria monocytogenes: a multifaceted model

Pascale Cossart[1]

[1]*Institut Pasteur, Cell Biology and Infection, Paris, France*

Intracellular pathogens are still responsible for many important public health problems. Their study as for extracellular pathogens allows to generate new tools for diagnostic, treatment or vaccines. But their intracellularity provides means to tackle host cell-specific mechanisms including signaling pathways, metabolism, cytoskeleton plasticity and many other aspects. Since 1986, how the intracellular bacterium *Listeria monocytogenes* survives in the environment and behaves during infection is the object of intense investigation. This bacterium is still responsible for severe food borne infections leading to gastroenteritis, meningitis and abortions, with a mortality rate of 30%. The recent epidemics in South Africa with ca. 200 deaths has demonstrated that epidemics can still occur and lead to an important number of deaths. The capacity of *Listeria* to produce an infection is due to its ability to cross three tight host body barriers: the intestinal barrier, the blood brain barrier, and the placental barrier. An arsenal of « virulence factors » allows *Listeria* to survive and persist in the intestinal lumen, to enter into cells and disseminate in the various tissues that it infects, exploiting cellular signaling pathways and components to its own profit. The talk will illustrate how investigating these various aspects have led to new concepts and changes in paradigms in several areas of biology. We will also present recent data, such as the interaction of *Listeria* with the gut microbiome and the role of several bacteriocins.

O003 Resource tracking and trade across complex symbiotic networks

Toby Kiers[1]

[1]*Vrije Universiteit Amsterdam, Ecological Science, Amsterdam, Netherlands*

Background: The arbuscular mycorrhizal symbiosis is characterized by complex underground networks of plants and fungi trading resources. While it has been shown that both fungal and plant individuals are able to preferentially allocate resources to higher quality partners, the trade dynamics governing these underground markets are unknown.

Objectives: How do hosts maintain cooperation with the most beneficial fungal symbionts over the course of evolution? How do fungi choose the most beneficial hosts? Adaptations that allow individuals to discriminate among partners based on actual symbiotic performance help promote the persistence of cooperation, and allow organisms to negotiate conditions of trade across complex networks.

Methods: My lab develops tools to visualize, track and quantify symbiotic trade over space and time. Our newest research tool utilizes high resolution imaging and video to track quantum-dot tagged nutrients across fungal networks and into host roots.

Results: Ultimately, we are interested in predicting how and when cooperation is favored to evolve across diverse symbiotic partnerships.

O004 The leaf microbiota: disassembling and rebuilding to explore plant microbe interactions

Julia Vorholt[1]

[1]*ETH Zurich, Institute of Microbiology, Zurich, Switzerland*

Background: The aerial parts of the plants, which are dominated by leaves, represent one of the largest terrestrial habitats for microorganisms. There is a growing interest to study commensal bacteria to elucidate their interactions with the plants, among each other and to learn how they withstand the hostile conditions of their habitat. Metagenomics and metaproteomics approaches gave insights into the community composition and general bacterial adaptation strategies to the phyllosphere.

Objectives: We conducted large-scale experiments to isolate Arabidopsis thaliana leaf bacteria as pure cultures to construct synthetic community experiments under gnotobiotic conditions.

Methods: Individual plants as well as individual leaves were sampled at different European sites to determine their core leaf community and to establish a reference strain collection using flow cytometry and dilution series plating. A representative strain collection covering the majority of species living in the phyllosphere of A. thaliana was established and draft genomes of all selected isolates (>200) were generated.

Results: Recolonization experiments using synthetic communities in a gnotobiotic model system showed reproducible colonization patterns and represent a valuable starting point to identify mechanisms of community formation and function. Examination of plant responses to its microbiota revealed that the plant reacts differently to members of its natural phyllosphere microbiota. A subset of commensals increase expression of defense-related genes and thereby contribute to plant health and performance.

O005 Plants as holobionts: New understandings, new opportunities

Philippe Vandenkoornhuyse[1]

[1]*University of Rennes 1, CNRS, UMR6553 Ecosystems Biodiversity Evolution, Rennes, France*

Animal and plants have been for a long time considered as standalone entities. However, animals and plants have never been alone. Research on microbiota have highlighted a common trend for both animals and plants, the tremendous importance of the symbiotic compartment on their host fitness.

From this knwoledge, a more holistic perception of what is a plant (or an animal) is necessary and the concepts of holobiont and hologenome have emerged allowing to rethink the individuality. The holobiont includes the host and its symbiotic compartment wathever the level of cooperation (i.e. from negative to positive interactions). The hologenome theory of evolution has been debeted mostly because of the limited number of studies focusing on the microbiota heritability.

For plants, proofs of transmission of a fraction of the host microbiota from a generation to the next are now available. This transmission can be vertical (i.e. from mother plant to seeds or to clones) or/and pseudo-vertical.

During the presentation I will develop from recently published papers how this holistic perception of plants viewed as holobionts is changing our understandings of plant fitness. I will discuss the microorganisms recruitment and transmission to the next generation and will discuss the consequences in terms of evolution.

New ideas are emerging from the concept of holobiont which could deeply change our understandings from which opportunities are raising for the next agriculture notably.

O006 Exercising influences: Distinct biotic interactions shape plant microbiomes

Sarah Lebeis[1], Sarah Stuart Chewning[2], David Grant[1], Bridget O'Banion[1], Katherine Moccia[1], Andrew Willems[1]

[1]*University of Tennessee, Knoxville, United States*
[2]*Yerkes National Primate Research Center, Atlanta, United States*

Background: While soils are an extremely diverse microbial environment, only a select and consistent set of microbial taxa colonize the endophytic compartment (EC) of roots, indicating that transition by microbes from life in the soil to the EC requires passing several levels of selection.

Objectives: Our studies explore microbial activities that facilitate robust colonization and selective pressures applied by either the host and established microbiota to gate subsequent root microbiome membership.

Methods: We use a large collection of bacterial isolates to build a synthetic community composed of a well-defined mixture of bacterial root isolates to inoculate several plant genotypes and nutrient levels. Genome comparisons of these bacteria uncovered common activities associated with robust colonizers of the root microbiome. Specifically, we use *Streptomyces* and *Pantoea* strains, which while low in relative abundance, are commonly found in many root systems.

Results: We reveal how their metabolic prowess directs the ability of *Streptomyces* to colonize *Arabidopsis thaliana* roots, as well as potentially influence abundance of other members of the root microbiome. In *Medicago sativa,* we investigate how the concentration of nutrients provided to plants correlates with the level of colonization of a non-pathogenic *Pantoea* strain. Further, we also determine the ability of this *Pantoea* to outcompete other bacterial isolates in a synthetic community in plants grown in varying levels of nitrogen concentration. Together, these studies allow us to begin to define the intersection of plant-microbe, microbe-microbe, and plant-environment interactions to result in community assembly and therefore function.

O007 Defining the host genetic control of the rhizosphere bacterial microbiota

Carmen Escudero Martinez[1], Rodrigo Alegria Terrazas[1], Senga Robertson-Albertyn[1], Rajiv Sharma[1], Davide Bulgarelli[1]

[1]*University of Dundee, Plant Sciences, Dundee, United Kingdom*

Background: My group uses barley (Hordeum vulgare) as a model to gain novel insights into the genetic basis of plant-microbiota interactions in the rhizosphere. We previously demonstrated that Elite varieties and wild barley ancestors host distinct microbiotas, possibly representing a footprint of plant domestication on the microbial communities inhabiting the rhizosphere.

Objectives: We recently extended this line of investigation by characterising the microbiota of a bi-parental population between an elite variety and a wild barley ancestor with the aim of identifying host genes regulating plant-microbe interactions in the rhizosphere

Methods: By combining 16S rRNA gene sequencing profiles, as 'quantitative traits', with thousands of SNPs in the barley genome we compiled a map of the plant loci shaping the rhizosphere microbiota.

Results: Strikingly, we did not observe a linear relationship between number of loci and bacteria putatively controlled by them. Rather, our data suggest that microbial community assembly in the barley rhizosphere is controlled by a few major alleles with a major effect. In particular, we identified a single locus on barley chromosome 3H significantly associated with the recruitment of nine, phylogenetically unrelated, bacteria. Here I will discuss the experiments that led us to these discoveries and their implications for basic and applied science.

O008 Microbiomes of hypersaline soils and salterns assessed by metagenomics

Antonio Ventosa[1], Blanca Vera-Gargallo[1], Ana Duran-Viseras[1], Rafael R. de la Haba[1], Cristina Sanchez-Porro[1]

[1]*Microbiology and Parasitology, Sevilla, Spain*

Background: Hypersaline habitats are extreme environments that have been widely used for microbiological studies. Besides their high salts content, other physicochemical features may limit the presence of microorganisms, such as temperature, pH, oxygen availability, pressure, etc… Thus, the microbes adapted to these habitats must cope with several factors and are considered as poly-extremophiles. They are represented by prokaryotes (archaea and bacteria) and some eukaryotes (protists and fungi).

Objectives: We will describe the prokaryotic microbial diversity of hypersaline habitats on the basis of metagenomic studies and very especially we will focus on the major differences found betweeen aquatic hypersaline systems (salterns) and hypersaline soils.

Methods: Metagenomic studies have been carried out on water samples obtained from ponds of salterns located in Santa Pola (Alicante) and Isla Cristina (Huelva) as well as on hypersaline soils from the Odiel saltmarshes area (Huelva), Spain.

Results: Hypersaline soils harbour a more diverse microbial community, comprising at least 29 different major phyla, in contrast to a lower number of phyla (up to 8 to 9) of ponds of Santa Pola saltern. The most abundant prokaryotes in the hypersaline water systems are members of the Euryarchaeota (haloarchaea), Bacteroidetes and Nanohaloarchaea. In the hypersaline soils studied we also determined the abundance of Euryarchaeaota and Bacteroidetes. Our studies suggest that some halophilic prokaryotes might be highly adapted to aquatic hypersaline habitats, as the square haloarchaeon Haloquadratum and the gammaproteobacterium Spiribacter. In contrast, microbes found in hypersaline soils have a more euryhaline response, showing a wider tolerance to salinity.

O009 High bacterial species novelty in metagenome assembled genomes (MAGs) from hot springs around the world (32 - 98 °C): metabolic insights of new phyla, classes and orders of thermophilic bacteria

Jaime Alcorta[1], Oscar Salgado[1], Tomás Alarcón-Schumacher[1;2], Sergio Guajardo-Leiva[1], Beatriz Díez[1;3]

[1]Pontificia Universidad Católica de Chile, Department of Molecular Genetics and Microbiology, Santiago, Chile
[2]Max Planck Institute for Marine Microbiology, Bremen, Germany
[3]Center for Climate and Resilience Research (CR)2, Santiago, Chile

Background: Hot spring microbial mats are composed by diverse bacteria and archaea whose presence and activity are modulated mainly by temperature, pH and geochemistry. The microbial diversity has been addressed lately by metagenomics to mainly report general patterns of taxonomy and metabolic processes. However, metagenome assembled genomes (MAGs) can be used to describe discrete genomic populations to improve our understanding of genomic adaptations of unknown/uncultured taxa that thrive in these extreme systems.

Objectives: Our aim is to study the taxonomy and codified metabolic processes from high quality MAGs obtained from 21 microbial mat Illumina metagenomes from Chile (unpublished) and public databases in a wide temperature range.

Methods: We analyzed 21 hot spring metagenomes within a sampling temperature range of 32 to 98 °C. All reads were quality trimmed and de-novo assembled. Contigs were binned into MAGs and their completness/contamination was analyzed with CheckM. High quality MAGs were taxonomically classified with GTDB-tk and their codified metabolic processes were annotated.

Results: We obtained 381 high quality MAGs with bacterial predominance (371). The taxonomic novelty of the obtained MAGs was across all levels, being classified in novel phyla (2), class (4), order (26), family (69), genus (171) and species (336). Therefore, a great species novelty (88%) whithin these 21 metagenomes was revealed. The metabolic processes encoded in these MAGs will improve our understanding of the roles of these uncultured microbes, such as the two MAGs from novel phyla potentially involved in nitrogen and methane biogeochemical cycles of these extreme systems.

O010 Brockarchaeota, a new archaeal phylum containing unique pathways for hydrogen-dependent methylotrophy

Valerie De Anda[1], Lin-Xing Chen[2], Brett J Baker[1], Nina Dombrowski[1], Zheng-shuang Hua[3], Hong-chen Jiang[4], Jillian F Banfield[2;5], Wen-jun Li[3]

[1]UT Marine Science Institute, Port Aransas, United States
[2]Earth and Planetary Sciences, University of California Berkeley, United States
[3]State Key Laboratory of Biocontrol and Guangdong Key Laboratory of Plant Resources, School of Life Sciences, Sun Yat-Sen University, China
[4]State Key Laboratory of Biogeology and Environmental Geology, China University of Geosciences, Wuhan, China
[5]Environmental Science, Policy and Management, University of California Berkeley, United States

Background: C1-methylated compounds are predominant in the oceans and the atmosphere, thus are crucial components of the global carbon cycle. The degradation of these compounds is an important source of methane in anoxic environments. However, the ability to metabolize C1-compounds without methane production has only been documented in two gut-archaeal species

Objectives: Here, we report the discovery of a new lineage of H_2-dependent methylotrophic archaea recovered from hot spring and deep-sea sediments, which has been overlooked in rRNA-based surveys. Phylogenomic analyses suggested this new lineage as a distinct phylum within the TACK superphylum

Methods: Surveys of public datasets revealed they are globally distributed in geothermal springs, thus we refer to them as Brockarchaeota, after Thomas Brock pioneer microbiologist for his groundbreaking research in hot springs. Metabolic inference of eight Metagenome-Assembled Genomes (MAGs) revealed several unique metabolic traits in Brockarchaeota

Results: Unlike other H_2-dependent methylotrophs, Brockarchaeota likely conserved the energy by electron bifurcation. They are also able to fix CO_2 via the Wood-Ljungdahl pathway. Brockarchaeota from deep sea sediments are able to utilize dimethylamine, while hot spring lineages are more versatile having pathways for the utilization of a variety of geothermally-derived substrates including arsenate, selenate and elemental sulfur, methanol, and trimethylamine. Comparative genomic analysis of the potentials in carbohydrates utilization indicated that Brockarchaeota could also play an important role in the breakdown of high molecular-weight plant-derived polysaccharides, primarily cellulose, hemicellulose and xylose. The broad distribution, and their unique carbon metabolism suggest that Brockarchaeota are key players in global carbon cycling.

O011 Prokaryotic diversity in terrestrial mud volcanoes of Taman peninsula, Russia

Alexander Slobodkin[1], Alexander Merkel[1], Galina Slobodkina[1], Nikolay Chernyh[1], Elizaveta Bonch-Osmolovskaya [1]

[1]*Winogradsky Institute of Microbiology, Research Center of Biotechnology of the Russian Academy of Sciences, Moscow,*

Background: Mud volcanism is one of the most exciting geological phenomena with significant implications in hydrocarbon exploration, seismicity, and atmospheric budget of methane. The presence of different inorganic and organic compounds, which can be used as electron donors and acceptors in microbial metabolism, allows the development of microorganisms with various physiologies.

Objectives: This study provides the first overview of bacterial and archaeal diversity in three terrestrial mud volcanoes (TMVs) located in the Taman peninsula, Russia obtained by means of high-throughput sequencing and presents the results on the isolation of pure cultures of cultivated anaerobes.

Methods: TMV microbial communities were studied using molecular (16S rRNA gene amplicons and metagenome sequencing) and cultural approaches.

Results: All investigated TMVs contained microorganisms associated with biogeochemical cycles of methane and sulfur. 16S rRNA gene sequences of Deltaproteobacteria and methanotrophic archaea were detected in all samples but differed at the genus and species levels between individual sites. One of the sites harbored the unique microbial community with high abundance of 16S rRNA gene sequences belonging to archaeal anaerobic methane oxidizers of ANME-3 group (39% of all sequences). This is the first report on detection of ANME-3 in terrestrial ecosystem and the first example of microbial community with dominance of ANME-3 group archaea in a total prokaryotic abundance. Five strains of anaerobic bacteria belonging to different physiological groups were isolated in pure culture. This study extends the current knowledge of the phylogenetic and metabolic diversity of microorganisms inhabiting terrestrial mud volcanoes.

O012 The Soil Resistome of a Natural Xeric Gradient in the Namib Desert

Yashini Naidoo[1], Rian Pierneef[2], Errol Cason[3], Angel Valverde Portal[3], Donald Cowan[1]

[1]Centre for Microbial Ecology and Genomics, Biochemistry, Genetics and Microbiology, Pretoria, South Africa
[2]Agricultural Research Council, Biotechnology Platform, Pretoria, South Africa
[3]University of the Free State, Microbial, Biochemical and Food Biotechnology, Bloemfontein, South Africa

Background: Recent studies have indicated that the wide-spread dissemination of antibiotic resistant determinants is largely associated with natural environmental reservoirs. This is because, the intensive use of antibiotics in animal farming and other agricultural practices results in the continuous release of antibiotics into the environment. This has led to the extensive occurrence of antibiotic resistance in both impacted and natural habitats. However, very little is known about the abundance and composition of antibiotic resistance genes (ARGs) in non-agricultural regions such as hot deserts.

Objectives: we aimed at 1) investigating the variation of ARG alpha diversity in Namib Desert soils and to examine how the composition (beta diversity) of ARGs varies in terms of changing environmental characteristics, 2) to assess whether composition and diversity of these ARGs relates to bacterial community composition and diversity and 3) to ascertain whether or not horizontal gene transfer affects the distribution of ARGs in this soil.

Methods: This study uses shotgun metagenomics and soil chemistry analysis across two different zones (low- and high-rain zone), over a period of two years, within a natural xeric gradient in the Namib Desert (n = 12; two zones x 3 samples x 2 years).

Results: Preliminary analysis reveals a total of 148 of ARGs, many of them encoding multi-drug efflux pumps. Resistance mechanisms involved in the inactivation of Fluoroquinolones and cephalosporins were present. Of the two factors taken into account (year, water regime), only year had an effect on alpha and beta diversity ($P < 0.5$ for both).

O013 Biodiversity and community composition of fungi associated to Biological Soil Crusts in Victoria Land, Antarctica

Fabiana Canini[1;2], József Geml[2], Luigi Paolo D'Acqui[3], Stefano Ventura[3;4], Silvano Onofri[1], Laura Selbmann[1;5], Laura Zucconi[1]

[1]*University of Tuscia, Department of Ecological and Biological Sciences, Viterbo, Italy*
[2]*Naturalis Biodiversity Center, Biodiversity Dynamics, Leiden, Netherlands*
[3]*National Research Council of Italy (CNR), Terrestrial Ecosystems Research Institute (IRET), Sesto Fiorentino, Italy*
[4]*The Italian Embassy in Israel, Tel Aviv, Israel*
[5]*Italian National Antarctic Museum (MNA), Mycological Section, Genova, Italy*

Background: Ice-free regions in Northern Victoria Land, Antarctica, are patchily distributed, mainly restricted to coastal regions and mountain peaks. In coastal areas, vegetation is composed only of lichens and mosses due to the extreme environmental conditions. The association of these life forms with soil particles and microorganisms (e.g., cyanobacteria, algae and microfungi) makes up biological soil crusts (BSCs), found worldwide in cold-dominated and/or arid and semi-arid regions, where plant growth is impaired. Despite BSC organisms being among the most ubiquitous life forms throughout ice-free areas of continental Antarctica, fungal communities that form a defining component of these ecosystems have never been widely investigated before.

Objectives: Main aims were to provide a first kingdom-wide characterization of fungal communities of these peculiar ecosystems and to understand how diversity and community composition are related to nutrient availability and different edaphic variables. Such analyses of these simplified ecosystems could provide a better understanding of how functional profiles of BSC fungal communities are related to abiotic factors.

Methods: We used DNA metabarcoding to compare the taxonomic and functional composition of fungal communities from 17 sites in 6 different localities from 73 to 77°S. Richness, community structure and functionality have been related to soil granulometry and chemical properties.

Results: Fungal richness was low (59 ± 27 OTUs per sample) and community composition was spatially auto-correlated. This was mainly driven by edaphic differences (as the presence of exchangeable cations etc.) among the sites and less strongly by possible differences in mesoclimatic conditions, as dispersal limitation is unlikely.

O014 Microbial biogeography and colonization of Arctic terrestrial ecosystems

Lucie Malard[1], David Pearce[1]

[1]*Faculty of Health and Life Sciences, Northumbria University, Newcastle Upon Tyne, United Kingdom*

Background: Identifying and understanding the ecological processes structuring microbial community assemblages is a central issue in microbial ecology. While biogeographical patterns of soil bacteria have been described on global scales, the Arctic region has been somewhat overlooked. Beyond spatial and environmental factors, the role of colonization on microbial community structure has yet to be determined.

Objectives: We characterized microbial communities of Arctic tundra soils from small to continental scales to identify biogeographical patterns, key environmental drivers and assessed the colonization potential.

Methods: Using X-Ray fluorescence and gravimetric measurements, we characterized elemental composition and physico-chemical properties of collected soils. Multivariate analyses, geostatistics and high throughput 16S sequencing were used to characterize microbial communities. Microcosms and in-situ experiments were conducted to assess the potential of snow microorganisms to colonize soil systems.

Results: By conducting a multi-scale Pan-Arctic survey of bacterial communities of Arctic soils, we identified biogeographic patterns and highlighted the role of geographical dispersal and environmental drivers in structuring microbial communities. We identified the Arctic soil core microbiome and indicated differences between Arctic soil communities and global communities. Overall, results highlight the heterogeneity of Arctic soils, the role of spatial and environmental factors on community structure and the potential for colonization. While this study brings a deeper understanding of Arctic microbial community assemblages, this is also a baseline for future functional studies in the region, which will be critical to forecast the ecological consequences of environmental change.

O015 Novel genomic determinants of respiratory metabolism in extremophilic prokaryotes

Sergey Gavrilov[1], Stepan Toshchakov[1], Dmitri Sorokin[1], Alexander Slobodkin[1], Ivan Elizarov[1], Olga Golyshina[2], Peter Golyshin[2], Michail Yakimov[3], Ilya Kublanov[1]

[1]Winogradsky Institute of Microbiology, Research Center of Biotechnology of the Russian Academy of Sciences, Moscow, Russian Federation
[2]Bangor University, School of Natural Sciences, Gwynedd, Bangor, United Kingdom
[3]Institute for Biological Resources and Marine Biotechnology, IRBIM-CNR, Messina, Italy

Background: Extremophilic prokaryotes are thriving at physico-chemical boundaries of life – temperatures up to 122°C, pH 0-12, 5-molar salinity. Many extremophiles represent deep phylogenetic lineages most closely related to the last common ancestor and could possess the most ancient as well as the most stable enzymes driving the key metabolic processes.

Objectives: Identification and description of novel determinants of anaerobic and aerobic respiration from extremophiles of various phylogenetic lineages.

Methods: Genomes of our several extremophilic isolates were screened for target genes using previously described strategies (doi:10.1111/1462-2920.12067); functions of target genes were evidenced at transcript or protein level, symbiotic interactions - by CARD-FISH.

Results: We have evidenced differential involvement of three novel *c*-type multihemes in Fe(III) respiration and electrogenesis of a thermophilic bacterium *Carboxydothermus ferrireducens*. For the first time, *c*-type multihemes determining Fe(III)-reduction were identified in hyperthermophilic archaea of *Pyrobaculum* genus. Three novel molybdopterin oxidoreductases were revealed in the genome of *Halanaeroarchaeum sulfurireducens*, determining sulfur respiration with acetate – a unique process for Archaea. Function of the enzymes was confirmed at transcript level. In a hyperacidophile *Cuniculiplasma divulgatum*, genes of A1-type heme-copper oxidase were identified. Phylogeny reconstruction and genomic context analysis put them into a separate cluster rooted to the ancestor form of all the modern A-type oxygen reductases. Genome analysis of putative *C.divulgatum*'s symbiont – a nanoarchaeon of 'DPANN' superphylum, revealed a cytochrome *bd*-oxidase to be its only candidate respiratory oxygen reductase, with the host cell being the most probable source of hemes and reduced quinones for the enzyme.

Funding: RSF project #17-74-30025.

O016 Integrative genomics sheds light on evolutionary forces that shape the extremely acidophilic lifestyle

Carolina González[1], Mark Dopson[2], Jorge Valdes[3], David S. Holmes[1;3]

[1]*Center for Bioinformatics and Genome Biology, Fundación Ciencia & Vida, Santiago, Chile*
[2]*Centre for Ecology and Evolution in Microbial Model Systems (EEMiS), Linnaeus University, Kalmar, Sweden*
[3]*Center for Genomics and Bioinformatics, Faculty of Sciences, Universidad Mayor, Santiago, Chile*

Background: Multiple phylogenomic approaches, suggest that the extremely acidophilic (<pH 3) genus *Acidithiobacillus* evolved from ancestral neutrophiles. The inferred evolutionary trajectory sheds light on how acid tolerance was acquired and highlights the gain, duplication and loss of functions associated with living at low pH, including amino acidic decarboxylation systems, proton exchangers as well as other pH homeostasis mechanisms.

Acidithiobacillus constitute a genus within the newly recognized *Acidithiobacillaceae* family. They have been isolated from a variety of extremely acidic habitats (< pH 3) around the world including acid mine drainages, natural acidic environments and industrial bioleaching operations. Acidithiobacilli are polyextremophilic chemolithoautotrophs, fixing CO_2 and N_2 from the atmosphere and using hydrogen, sulfur and, in some cases, iron as energy sources.

Methods: A comparison of the predicted core- and pan-genomes of Acidithiobacilli with their inferred last common ancestor, provides insight into the several evolutionary strategies that allowed the genus to transition from a neutral to an acidic environment (pH-ladder hypothesis), including gene gains by horizontal gene transfer, gene losses, mutation and gene redundancy.

Results: Three highlights of the results are the gain of genes encoding a hopanoid pathway for membrane stabilization at low pH, an initial adaptation in a moderately thermal environment that may facilitate the final transition into an acidic environment and the presence of a large pool of accessory genes with functional redundancy that provides the opportunity to “hedge bet” in rapidly changing acidic environments.

O017 Digitalizing heterologous gene expression in Gram-negative bacteria with portable ON/OFF gadgets

Belen Calles[1], Angeles Hueso[1], Angel Goni-Moreno[2], Victor de Lorenzo[1]

[1]*CNB-CSIC, Madrid, Spain*
[2]*Newcastle University, School of Computing, Newcastle, United Kingdom*

Background: Although prokaryotic promoters controlled by signal-responding regulators typically display a range of input/output ratios when exposed to cognate inducers, virtually no naturally occurring cases are known to have an off state of zero transcription—as ideally needed for synthetic circuits.

Objectives: To overcome this problem we have modelled and implemented simple digitalizer module that entirely suppresses the basal level of otherwise strong promoters so that expression in the absence of induction is completely impeded.

Methods: The thereby designed circuit involves the interplay of a translation-inhibitory sRNA with the translational coupling of the gene of interest to a repressor such as LacI. The digitalizer module was validated with the strong inducible promoters Pm (induced by XylS in the presence of benzoate) and PalkB (induced by AlkS/dicyclopropylketone) and shown to perform effectively both in *E. coli* and the soil bacterium *Pseudomonas putida*. The entire circuit was rigorously described with the synthetic biology open language (SBOL) format.

Results: The distinct expression architecture of the device allowed cloning and conditional expression of eg colicin E3, one molecule of which per cell suffices to kill the host bacterium. Revertants that escaped ColE3 killing were not found in hosts devoid of insertion sequences, suggesting that mobile elements are a major source of circuit inactivation *in vivo.* Interfacing the digital switch with a light-controlled expression system and/or hyperactive cdGMP cyclase enabled stringent control of biofilm formation in *P. putida*. The device was instrumental also to create switches for changing the gross physiological status of cells.

O018 High-throughput interaction profiling in bacteria

Athanasios Typas[1]

[1]*European Molecular Biology Laboratory, Genome Biology Unit, Heidelberg, Germany*

Systematic and quantitative profiling of functional interactions at a genome-wide level provides unique insights into cellular behaviors and the underlying network architecture. We have developed new tools and strategies for such systematic approaches in bacteria. Here, I will present recent developments on this front, and provide insights into how these approaches can be used to uncover new biology in different bacterial species, and their interfaces with other microbes, the host and the environment.

O019 Regulation of cell elongation and division under fluctuating resource constraints

Kerwyn Casey Huang[1]

[1]*Stanford University, Department of Bioengineering, Standford, United States*

Bacterial cells regularly face environmental variation in their natural habitats, and need to respond robustly and quickly to those fluctuations. In the rod-shaped bacterium Escherichia coli, steady-state cell shape has been shown to change in response to different nutrient conditions, through regulations of both cell elongation and division. However, it remains unclear how cells respond to continuous environmental changes and regulate cell shape. In this work, we develop a time-delay model that fully explains the dynamics of surface area and volume synthesis in a laboratory batch culture, which shows a universal set of dynamics of surface area to volume ratio (SA/V) for single-cell organisms. This model also predicts that SA/V is robust to division perturbations, but systematically changes with cell wall synthesis rates and translation rates, all of which have been experimentally verified. We then develop a mathematical model that quantiatively predicts the variation in cell shape across mutants based on shape-dependent changes to cell-cycle variables. Taken together, our work provides a quantitative framework for understanding cellular resource allocation, and has implications for how cells coordinate elongation and division under global resource constraints.

O020 Tools for engineering coordinated system behavior in synthetic microbial consortia

Karen Polizzi[1]

[1]*Imperial College London, Chemical Engineering, London, United Kingdom*

Background: In Nature, microbial communities live in close proximity and often work together to degrade complex substrates. In synthetic biology, we wish to emulate this behavior by dividing complex gene circuits across multiple populations. Engineering synthetic microbial communities requires multiple orthogonal cell-to-cell communication channels to propagate information with minimal signal interference. Here we focus on quorum sensing, a natural bacterial mechanism for coordinating population behavior.

Objectives: The objectives of this work were to build a toolbox to enable the rapid identification of orthogonal communication channels for building synthetic microbial consortia.

Methods: 6 acyl homoserine lactone receiver devices were constructed and characterized by flow cytometry. The input-output responses were fit with a simple logistic model to capture key variables such as basal and maximal activation, EC50, and sensitivity. We used this information to develop a software tool that allows automated selection of orthogonal chemical channels. We then used the tool to identify and build a system using three orthogonal channels simultaneously.

Results: We characterized a library of 6 acyl homoserine lactone receiver devices, quantifying all cognate and non-cognate chemical signal interactions. We subsequently developed a software tool for the design of consortia that can flexibly accommodate user-defined constraints for fold changes in gene expression and identify any number of desired communication channels. Furthermore, we experimentally validated one of the software predictions by engineering a polyclonal co-culture capable of controlling gene expression using three non-interfering AHL communication channels.

O021 From understanding to application of bacterial chemotaxis

Victor Sourjik[1]

[1]*Max Planck Institute for Terrestrial Microbiology, Marburg, Germany*

Background: Chemotaxis is one of the best-studied bacterial behaviours, but its significance for physiology and ecology of bacteria are only poorly understood.

Objectives: Our aims were first to systematically investigate the sensory spectrum of chemotaxis of the model organism *Escherichia coli*, as well as of other bacteria, and second to utilize chemotactic bacteria as delivery systems for microscopic cargo particles.

Methods: We have utilized FRET-based and microfluidic assays to quantify the chemotactic response to a wide range of chemical and physical stimuli, and cell tracking and microfabrication to study the capability of bacteria to transport cargo of different sizes.

Results: We show that *E. coli* chemoreceptors can detect a wide range of stimuli, including not only nutrients but also human hormones, temperature, osmolarity, aromatic compounds, and quorum signaling molecules, using a number of distinct mechanisms of sensing. We have further developed approaches that allow rapid and high-throughput characterization of ligand specificities of chemoreceptors from different species. Finally, we showed that *E. coli* can be efficiently loaded on micrometer-sized particles or eukaryotic cells and used to transport them in microfluidic channels.

O022 Defining patterns in chemical and genomic space to prioritise antibiotic discovery

Sylvia Soldatou[1], Grimur Hjorleifsson Eldjarn[2], Justin van der Hooft[3], Simon Rogers[2], Katherine Duncan[4]

[1]University of Aberdeen, Department of Chemistry, Aberdeen, United Kingdom
[2]University Of Glasgow, School of Computer Science, Glasgow, United Kingdom
[3]Wageningen University, Department of Bioinformatics, Wageningen, Netherlands
[4]University of Strathclyde, Strathclyde Institute of Pharmacy and Biomedical Sciences, Glasgow, United Kingdom

Background: Microbial drug discovery in the 'omics era relies on three key datasets, biosynthetic, chemical and biological (activity). Yet, to integrate and interrogate these large and complex datasets remains a challenge and results in the low-throughput prioritization of only a few strains based on observed antibiotic activity. Despite this wealth of genomic and metabolomic data, linking metabolites to the BGC responsible for their production and to observed bioactivity is limited, slow (manual) and challenging. Furthermore, our current discovery is reliant on existing biosynthetic, chemical and antibiotic knowledge, thus overlooking unidentified parent ions (metabolites) or hypothetical proteins (BGCs) which is the exact chemical and biosynthetic space which should be prioritized to identify novel antibiotics.

Objectives: Here, approaches to combine data sets consisting of bacterial genomes (and their predicted BGCs), the chemical products of these same strains and their bioactivity profiles will be discussed.

Methods: A dataset of Actinobacteria genomes have been mined for BGCs and these strains fermented to generate metabolite extracts for comparative metabolomics (high resolution tandem mass spectrometry / molecular networking) and antibiotic screening (against a panel of clinically relevant pathogens).

Results: A dataset of 25 Polar rare-actinomycete genomes with almost 200 BGCs, combined with 100 metabolite profiles (each strain under four fermentation conditions) was generated. Machine learning tools have been developed to establish patterns across strains and learn relationships between BGC, spectral features and bioactivity.

O023 Computational Tools for Prioritizing Drug Discovery in Actinomycetes

Nadine Ziemert[1]

[1]*University of Tuebingen, Interfaculty Institute of Microbiology and Infection Medicine, German Centre for Infection Research (DZIF), , Germany*

Next generation sequencing methods have made sequencing faster, cheaper, and easier than ever and have revolutionized almost every field of biology. The constantly growing volume of DNA sequence data has made genome mining an important tool for the detection and prediction of promising secondary metabolites and has lead to a renaissance in natural product based drug discovery. Thousands of putative gene clusters are available in public databases such as NCBI and JGI-ABC, the challenge being now to triage the most promising pathways to guide laborious wet-lab experiments, assist with the dereplication of already known compounds and predict interesting bioactivities based on genomic data.

Here we introduce a selection of computational tools developed by the Ziemert lab that are based on the evolution of secondary metabolites and can be used for a rapid automated identification and examination of novel biosynthetic gene clusters. We highlight first results of mining rare and underexplored actinomycete genera for promising natural compounds.

O024 Cheese-rind microbes: Untargeted metabolomics of complex biological systems

Laura Sanchez[1], Jessica Cleary[1], Gordon Luu[1]

[1]*College of Pharmacy, Chicago, United States*

Background: Multispecies microbiome systems have recently been closely linked to human, animal, and plant life processes. The growing field of metabolomics presents the opportunity to detect changes in overall metabolomic profiles of microbial species interactions. These metabolomic changes provide insight into function of metabolites as they correlate to different species presence and the observed phenotypic changes, but detection of subtle changes is often difficult in samples with complex backgrounds.

Objectives: Natural environments such as soil and food contain many molecules that convolute analyses, and identification of microbial metabolites amongst environmental metabolites is an informatic problem we begin to address here. Our microbes are derived from naturally aged cheese and grown on solid or liquid cheese curd media for the purpose of identifying metabolites involved in bacterial-fungal interactions. This medium, which is necessary for proper microbial growth, contains high amounts of salts, lipids, and casein breakdown products which make statistical analysis using mass spectrometry data difficult due to the high background from the media.

Methods: LC-MS/MS and MALDI-TOF biological and technical replicates from cheese derived microbial species were collected and the algorithm BLANKA was written in python.

Results: We have developed a simple algorithm to carry out background subtraction from microbes grown on solid or liquid cheese curd media to aid in our ability to conduct statistical analysis so that we may prioritize metabolites for further structure elucidation. The resulting data sets are statistically analyzed using the online MetaboAnalyst platform and significant metabolites will be prioritized.

O025 Proteome organization in microbes: genetic control and phenotypic efforts

Ruedi Aebersold[1]

[1]*Institute of Molecular Systems Biology, Biology, Zurich, Switzerland*

Background: In microbes and eukaryotes alike, phenotypes arise from the biochemical state of cells which, in turn, are the result of the composition of biomolecules, their interactions and their organization in the cell. Over the last two decades, a range of technologies has been developed to study biochemical processes as complex systems via the comprehensive, quantitative measurement of different classes of biomolecules. Due to the robustness and easy access of the corresponding technologies, systems microbiology studies have been based on genomic measurements, yet the intricacies of biological systems cannot be fully explained by genomic data.

Objectives: We will discuss SWATH-MS, a data independent MS-based proteomic technique, and computational resources to quantify essentially all expressed proteins in microbial cells with a high degree of accuracy and reproducibility. The method has reached a state where thousands of proteins can be quantified from a microgram of total peptide mass, at CV's of 10% and at a throughput of 60 samples per day on a single instrument, thus making large scale systems studies a reality.

Methods: We will further discuss an extension of the SWATH-MS method that allows us to detect and quantify hundreds of protein complexes from native protein extracts, thus indicating the organizational state of the proteome and changes thereof.

Results: The capacity of this technique to provide new insights into biochemical systems in microbes will be demonstrated with selected applications. Overall, we will show that advanced proteomic technologies have become an indispensable component of the tool set of systems microbiology.

O026 An automatically reconstructed model of bacterial gene regulation enables simulation, prediction, and perturbation of gene responses

Rodrigo Santibáñez[1;2], Daniel Garrido[2], Alberto Martin[1]

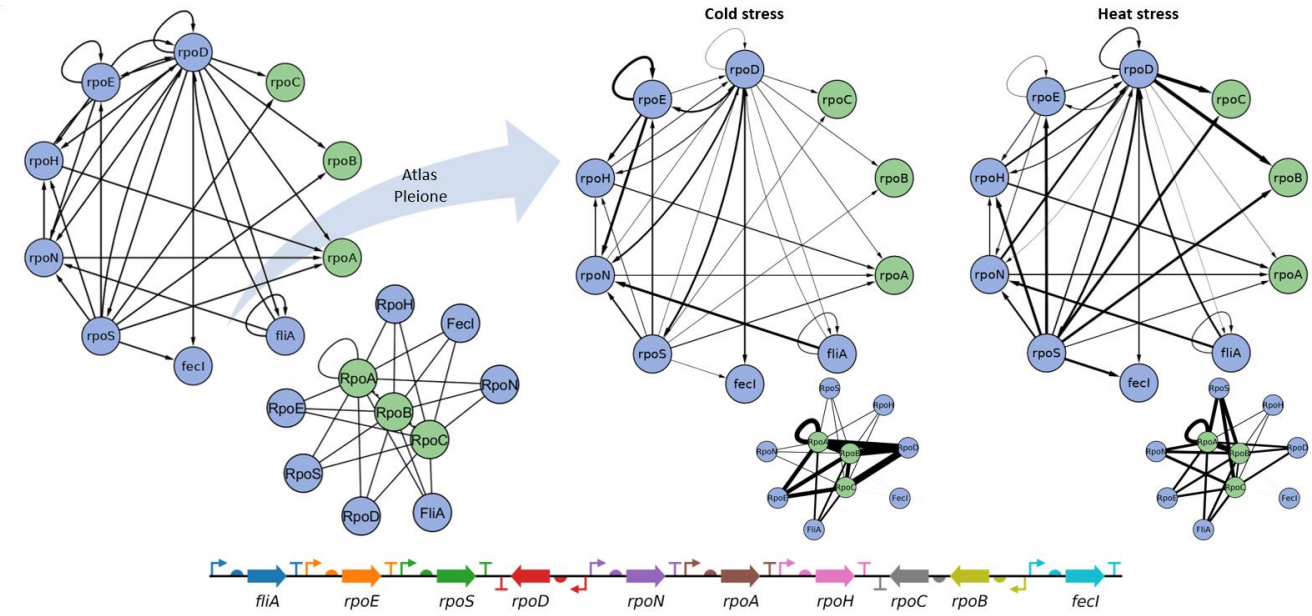
[1]*Universidad Mayor, Centro de Genómica y Bioinformática, Huechuraba, Chile*
[2]*Pontificia Universidad Católica de Chile, Department of Chemical Engineering and Bioprocesses, Santiago, Chile*

Background: Regulation of gene expression is essential for the working of any cell, allowing homeostasis of the internal state and adaptation to variations in the environment. This regulation relies on a network of transcription factors and other proteins that interact with promoters to trigger a genetic program. However, the complexity of the network precludes efforts to model gene regulation at genome-scale.

Objectives: To automate the reconstruction of a computational model from a bacterial transcriptional regulatory network (TRN). In addition, includes other biological networks, such as metabolic and protein-protein and regulatory RNAs-mRNA interactions, completing in this way the landscape of phenomena that control all events within a bacterial cell. Also, to analyze our model and study other aspects of gene expression, including knockouts (KOs), and other genetic modifications.

Methods: We developed Atlas, a tool that converts a static network into a Rule-based model. Atlas identifies the components and interactions, and writes proper equations. We also developed Pleione to calibrate Rule-based models using a statistical fitness function. We employed as an example the *Escherichia coli* TRN and microarray data to calibrate the models produced by Atlas.

Results: We validated Atlas and Pleione employing the sigma factors TRN. This network includes the seven sigma factors and the three RNA polymerase genes. We calibrated the model employing microarray data of cold and heat stresses, showing differential use of sigma factors. Single KOs showed that no sigma factor is essential for these genes. We developed a larger 1850-genes model from the RegulonDB TRN.


Cold stress
Heat stress
Atlas
Pleione
rpoD
rpoE
rpoC
rpoH
rpoB
rpoN
rpoA
rpoS
fliA
fecI
RpoH
FecI
RpoA
RpoN
RpoE
RpoB
RpoC
RpoS
RpoD
FliA
fliA
rpoE
rpoS
rpoD
rpoN
rpoA
rpoH
rpoC
rpoB
fecI

O027 Empowering Pseudomonas putida with surface-displayed designer protein scaffolds

Pavel Dvorak[1], Victor de Lorenzo[2], Esteban Martínez-García[2]

[1]Masaryk University, Microbiology Section, Department of Experimental Biology, Brno, Czech Republic
[2]Spanish National Centre for Biotechnology (CNB-CSIC), Synthetic and Systems Biology Program, Madrid, Spain

Background: *Pseudomonas putida* KT2440, the best-characterized and safe pseudomonad, belongs among the most promising bacterial hosts for synthetic biology and biotechnology endeavours. *P. putida*-based applications would greatly benefit from novel catalytic activities displayed on its surface. However, recombinant protein secretion in this G- host is inefficient and not well established.

Objectives: We sought to meet this challenge by developing a system for efficient display of recombinant proteins on *P. putida* surface by employing a synthetic biology approach.

Methods: New engineered *P. putida* strains EM42 and EM371, with reduced genomes and altered physiological properties, were combined with surface display of small anchoring protein scaffolds inspired by natural cellulosomes. These synthetic scaffolds served as docks for recombinant proteins tagged with complementary binding domains. Attachment of the proteins to the *P. putida* surface was verified by enzyme assays, confocal microscopy, fluorescence spectroscopy, and flow cytometry.

Results: Synthetic scaffolds containing one or two cohesin binding domains were successfully displayed on the surface of both *P. putida* strains with one of four tested autotransporter systems. Beta-glucosidase and two different fluorescent proteins were anchored to the surface of *P. putida* EM42 and EM371 with high efficiency especially in the latter case. This study shows the benefits of the strain EM371 for the surface display of recombinant proteins and introduces designer cellulosome strategy tailored for *P. putida*.

O028 Playing with the rhizobial Mega- Apps: creation and characterization of a genomically hybrid strain in the nitrogen-fixing symbiotic bacterium Sinorhizobium meliloti

Alice Checcucci[1], George diCenzo[1], Veronica Ghini[2], Marco Bazzicalupo[1], Anke Becker[3], Francesca Decorosi[4], Johannes Doehlemann[3], Camilla Fagorzi[1], Turlough Finan[5], Marco Fondi[1], Claudio Luchinat[2;6], Paola Turano[2;6], Tiziano Vignolini[7], Carlo Viti[4], Emanuele Biondi[8], Alessio Mengoni[1]

[1]*University of Florence, Department of Biology, Sesto Fiorentino, Italy*
[2]*CERM & CIRMMP, University of Florence, Sesto Fiorentino, Italy*
[3]*LOEWE-Center for Synthetic Microbiology, Marburg, Germany*
[4]*University of Florence, Department of Agri-food Production and Environmental Science, Florence, Italy*
[5]*McMaster University, Department of Biology, Hamilton, Ontario, Canada*
[6]*University of Florence, CERM and Department of Chemistry, Sesto Fiorentino, Italy*
[7]*European Laboratory for Non-Linear Spectroscopy, LENS, Sesto Fiorentino, Italy*
[8]*CNRS-Aix-Marseille University, LCB, Marseille, France*

Background: Many rhizobia harbour a multipartite genome composed by more than one replicon. Symbiotic and nitrogen-fixing phenotypes are mainly encoded by genes residing on the secondary replicons. Approaches for modifying such phenotypes may involve large-scale genome manipulation of secondary replicons and eventually their mobilization among different hosts to obtain genomically-hybrid strains with improved symbiotic performances.

Objectives: Here we report the creation and the multi-omics characterization of a genomic hybrid strain in a model multipartite genome species, the plant-symbiotic bacterium *Sinorhizobium meliloti.*

Methods: The secondary replicon pSymA (accounting for nearly 20% of total genome content and containing 1300 genes) was mobilized from a donor *S. meliloti* strain to an acceptor strain lacking pSymA. The hybrid strain was screened for a panel of complex phenotypes (carbon/nitrogen utilization phenotypes, NMR metabolomics on intra- and extra-cellular environment, symbiotic behaviour and transcriptional profile through RNA-sequencing). Additionally, metabolic network reconstruction and constraint-based modelling were employed for in silico prediction of metabolic flux reorganization.

Results: Phenotypes of the *cis*-hybrid strain were in good agreement with those of both parental strains. Interestingly, the symbiotic phenotype showed a marked cultivar-specific improvement with the hybrid strain compared to both parental strains. These results provide a proof-of-principle for the feasibility of genome-wide replicon-based remodelling of rhizobial strains for improved biotechnological applications in precision agriculture.

O029 The road to the enrichment of specialized N2O-reducers in an open mixed culture

Francesc Corbera-Rubio[1], Nina Roothans[1], n de Jonge[2], j Lund Nielsen[2], m Pabst[1], David Weissbrodt[1], Mark van Loosdrecht[1], Michele Laureni[1;2]

[1]Delft University of Technology The Nehterlands, Department of Biotechnology, Delft, Netherlands
[2]Aalborg University, Department of Chemistry and Bioscience, Aalborg, Denmark

Background: Nitrous oxide (N_2O) has a global warming potential 300 times higher than CO_2, and accounts for 8% of anthropogenic GHG emissions. The majority of N_2O results from microbial activity in agricultural soils and wastewater treatment plants. While multiple processes lead to N_2O formation, the reduction of N_2O to innocuous N_2 – the last step of the denitrification pathway – is the only known microbial N_2O sink. As such, denitrification holds promise to play a pivotal role in global efforts to contrast N_2O emissions.

Objectives: In this work, we focus on denitrification and in particular on N_2O-reducing microorganisms that lack the genetic potential to produce N_2O and thus represent a pure N_2O-sink. Specialist N_2O-reducers have been recently shown to be highly abundant in different ecosystems, yer the conditions selecting for them remain largely unknown.

Methods: To this end, two 2-L continuously-fed reactors were run with N_2O and acetate as sole electron acceptor and donor. Microfiltration membranes were used for cultivation over a broad range of solid retention times (SRT; 1-30 days), as encountered in biofilms and sediments.

Results: Operation under acetate-limiting conditions and at an SRT of 7 days resulted in the reproducible selection of specialized N_2O-reducers, as supported by the progressive decrease in biomass specific NO_2^- reduction rate. The meta-genome and meta-proteome of the enrichment are currently being analyzed for complete phylogenetic and functional characterization of the selected organisms. Ultimately, this is the first study reporting the enrichment of specialized N_2O-reducers in open mixed-communities.

O030 The multi-level relationship between heterotrophic bacteria and nutrients

Elena Perrin[1], Paola Turano[2], Veronica Ghini[2], Michele Giovannini[1], Barbara Cardazzo[3], Lisa Carraro[3], Ermenegilda Parrilli[4], Maria Luisa Tutino[4], Renato Fani[1], Marco Fondi[1]

[1]*University of Florence, Dep. of Biology, Firenze, Italy*
[2]*university of florence, CERM, Firenze, Italy*
[3]*University of Padua, Dipartimento di Biomedicina Comparata e Alimentazione, Legnaro, Italy*
[4]*University of Naples, Dipartimento di Scienze chimiche, Naples, Italy*

Background: A detailed comprehension of the functioning of heterotrophic bacteria metabolism is crucial for deciphering the complex processes that regulate nutrients assimilation and recycling in the oceans and the possible effect of external perturbations on such a system.

Objectives: We want to disclose the molecular details of two main features in the relationship between marine microbes and nutrients: 1) the formation of microbial aggregates in response to external nutrients concentration, 2) the regulation of nutrient assimilatory pathways when facing a nutritionally rich environment.

Methods: We combined a series of transcriptomics and metabolomics experiments in the model heterotrophic bacterium *Pseudoalteromonas haloplanktis* TAC125. We made use of mathematical modelling to generate predictive models and to integrate -omics data.

Results: Nutrients concentration in the medium deeply influences cell aggregation and (partially) cell motility in *P. haloplanktis* TAC125. Cells grown in a nutrient-rich medium appeared mucoid, non-motile and overall displayed a greater tendency to aggregate in respect to cells grown in a nutrient poor medium. Transcriptomics analysis was carried out in such conditions, revealing the gene clusters likely involved in such processes and a possible regulatory circuit accounting for the switch between planktonic and aggregated cells. Time-course transcriptomics on cells grown in a nutritionally complex environment revealed a time-resolved hierarchy in the metabolism of available nutrient sources, with metabolic switches marking the boundaries of each of the assimilated set of compounds. The combination of targeted metabolomics and mathematical modelling allowed the formulation of a computational model capable of explaining and predicting such metabolic phenotypes.

O031 Pooled Sequencing Enables High-Throughput Synthetic Genetic Circuit Characterisation

Matthew Tarnowski[1], Thomas Gorochowski[2]

[1]*University of Bristol, Biological Sciences, Bristol, United Kingdom*
[2]*University of Bristol, Biological Sciences, United Kingdom*

Background: Biological engineering requires well characterised genetic parts in order to predictably design and assemble synthetic genetic circuits (SGCs). Genetic part function is highly dependent upon local genetic context, making the function of SGCs containing new genetic part combinations unpredictable. Until recently SGCs could only be characterised slowly, indirectly and on an individual basis, using assays with fluorescent reporter protein readouts. The purpose of our study was to demonstrate that large pools of SGC variants can be characterised simultaneously, in high-throughput, using long read sequencing.

Objectives: Our objectives were two-fold: (1) long read sequencing based characterisation of a pooled SGC library and (2) characterisation of intrinsic transcription terminator dependence upon local genetic context.

Methods: Using one-pot pooled ligation we assembled a library of 360 SGCs with systematically varied terminator context. This pooled SGC library was assayed *in vitro* using long read RNA sequencing to simultaneously characterise each terminator variant.

Results: Our results illustrate that pooled assembly and pooled sequencing offer a high throughput method to build and characterise SGCs which elicit a sequenceable readout. This study offers a prototype method for rapid and thorough characterisation of both genetic parts and SGCs. Using this approach, myriad future microbial biotechnology applications can be developed.

O032 Bacterial autotrophy: where are we now? A state-of-the-nation overview and consideration of what physiology and biochemistry can we understand from geochemical, thermodynamic and physical parameters from an organism's habitat

Rich Boden[1]

[1]*University of Plymouth, School of Biological and Marine Sciences, PLYMOUTH, United Kingdom*

Background: Bacterial autotrophy has been observed for hundreds of years – with many phototrophic *Bacteria* from the class "*Cyanobacteria*" originally identified as plants or as algae, followed by the observation of sulfide, ammonia and nitrite oxidation by chemolithoautotrophs by Winogradsky. Our understanding of the carbon assimilation by autotrophs currently spans the Calvin-Benson-Bassham cycle (with two variants, one in the "*Cyanobacteria*" and *Eukarya* and one in other *Bacteria* – both photoautotrophs and lithoautotrophs), the Arnon-Buchanan cycle (*e.g.* in *Chlorobaculum* spp.), the Wood-Ljungdahl pathway (*e.g.* in *Clostridium* spp.) and the Holo-Sirevåg-Fuchs bicycle (*e.g.* in *Chloroflexus* spp.), with the Fuchs pathway found in the *Archaea* (*e.g. Metalosphaera* spp.). Intimately intwined in the assimilatory abilities, needs and regulation are the energy conservation pathways, which have great diversity both in terms of photoautotrophs with their multiple photosytems and other light-harvesting complexes and the lithoautotrophs with their myriad electron donors, including elementary sulfur, polythionates, molecular hydrogen, arsenite, ammonia, nitrite, ferrous iron *etc*. To fully understand the physiology of the autotroph, we must return to its environment and consider the geochemical, thermodynamic and physical factors that dictate which pathways, transporters, carboxysomes *etc* are required for growth.

Objectives: In this presentation I give an overview of the state of the nation regarding research in autotrophy and allied energy conservation pathways, which will also introduce our other speakers in the Autotrophy session. I will also present some novel findings.

Methods: Geochemical modelling and thermodynamic methods are used to consider the needs of the autotroph *in situ*.

Results: N/A

O033 Surprising heterogeneity in mechanisms for inorganic carbon uptake and fixation by autotrophic gammaproteobacteria from hydrothermal vents and other sulfidic habitats

Kathleen Scott[1], Tara Harmer[2], Rich Boden[3]

[1]*University of South Florida, Integrative Biology, Tampa, United States*
[2]*Stockton University, Biology Program, Galloway, United States*
[3]*University of Plymouth, School of Biological & Marine Sciences; Sustainable Earth Institute, Plymouth, United Kingdom*

Background: Six autotrophic carbon fixation pathways are known among *Bacteria* and *Archaea*. The 'front-end' of these pathways, delivery of dissolved inorganic carbon (DIC; = CO_2 + HCO_3^- + CO_3^{2-}) from the environment to the cytoplasm, has only been described in "*Cyanobacteria*", in which CO_2 concentrating mechanisms (CCMs), consisting of DIC transporters and carboxysomes, facilitate growth under low-DIC conditions. CCMs are likely to be common beyond the "*Cyanobacteria*", since concentrations of DIC vary among habitats, and could impact rates at which autotrophic *Bacteria* and *Archaea* catalyze reactions of geochemical importance.

Objectives: The objective of this study was to characterize CCMs from members of *Hydrogenovibrio, Thiomicrospira* and *Thiomicrorhabdus,* ubiquitous *Gammaproteobacteria* that fix carbon at hydrothermal vents, coastal sediments, hypersaline lakes, and other sulfidic habitats.

Methods: The genomes of these organisms were sequenced to find potential CCM components. qRT-PCR was used to determine whether genes were upregulated under low-DIC conditions. Potential DIC transporters were heterologously expressed in *E. coli* to measure DIC uptake activity.

Results: Carboxysome loci are present in the genomes of most of these organisms. Downstream of the carboxysome loci, genes encoding potential transporters are present, encoding members of four distinct transporter families. These genes were upregulated in cells grown under low-DIC conditions, and, when expressed in *E. coli,* demonstrated DIC uptake activity. This expansion in known DIC transporters across four families, from organisms from a variety of environments, provides insight into the ecophysiology of autotrophs, as well as a toolkit for engineering microorganisms for carbon-neutral biochemistries of industrial importance.

O034 Adaptation to the energy landscape in photoautotrophic bacteria

Thomas Hanson[1], Amalie Levy[2], Jacob Hilzinger[1], Katie Kalis[1]

[1]University of Delaware, School of Marine Science and Policy/Delaware Biotechnology Institute, Newark, United States
[2]University of Delaware, Chemical and Biomolecular Engineering/Delaware Biotechnology Institute, Newark, United States

Background: The phototrophic green sulfur bacteria, the *Chlorobiaceae*, fix CO_2 via the reductive TCA cycle using reducing equivalents derived from the oxidation of reduced sulfur compounds. These organisms are obligate autotrophs, but some species assimilate simple organic compounds like acetate and pyruvate. The *Chlorobiaceae* are considered to be physiological specialists, with a limited repertoire of regulatory machinery.

Objectives: The *Chlorobiaceae* grow on very low light levels relative to other photoautotrophic bacteria. Part of this capability is due to their exquisite antenna complex, the chlorosome. We hypothesize that other adaptive responses contribute to their ability to grow autotrophically in the face of variations in electron donor quality, fixed carbon availability, and light intensity that shape their energy landscape

Methods: A combination of classical growth and composition analyses were employed alongside transcriptome and methylome analysis to assess how *Chlorobaculum tepidum*, an established model for the *Chlorobiaceae*, and *Prosthecochloris* sp. strain CB11 adapted to varying energy availability.

Results: The data indicate that both *Cba. tepidum* and *Prosthecochloris* sp. strain CB11 utilize multiple strategies ranging from transcriptional regulation to genome methylation to adapt to changes in the energy landscape. In the case of *Cba. tepidum*, this leads to biomass composition reflecting energy availability, which has implications for metabolic modeling. The potential for understanding and controlling these adaptive responses enabling autotrophic biotechnology based on the consumption of toxic reduced sulfur compounds will also be discussed.

O035 Life on the (far-red) edge of darkness: how terrestrial cyanobacteria photoacclimate to and utilize far-red light for oxygenic photosynthesis

Donald Bryant[1]

[1]*The Pennsylvania State University, Dept of Biochemistry, University Park, PA, United States*

Background: Terrestrial cyanobacteria that grow in soil, microbial mats or plant shade occur in niches highly enriched in far-red light (FRL). In response to wavelengths longer than ~700 nm, some terrestrial cyanobacteria undergo an extensive photoacclimation response, FRL photoacclimation (FaRLiP).

Objectives: Characterize the FaRLiP response

Methods: Modern microbial physiological methods.

Results: RfpA, a knotless red/far-red phytochrome/histidine kinase, RfpC, a CheY-like phosphate shuttle, and RfpB, a response regulator/transcriptional activator, control the expression of a highly conserved, 20-gene cluster. Expression of the FaRLiP genes causes extensive remodeling of Photosystem I (PSI), PSII, and phycobilisomes. FaRLiP gene products replace the paralogous core subunits of these complexes produced when cells are grown in white light (WL). Cells grown in FRL additionally synthesize chlorophyll (Chl) *f,* Chl *d*, and special phycobiliproteins, all of which contribute to enhanced FRL absorption. The FRL-PSI complexes still employ a heterodimer of Chl *a*/Chl *a´* as the special pair (P700). The PSII complexes produced in FRL contain Chl *d*, Chl *f*, Chl *a*, pheophytin *a* and β-carotene. One of the genes in the FaRLiP gene cluster, a super-rogue paralog of *psbA* of PSII now known as *chlF*, encodes Chl *f* synthase. ChlF forms homodimers similar to the core heterodimer of PSII and is a photo-oxidoreductase that acts on Chl *a* or Chlide *a*. The discovery of this new type of photooxidoreductase has important implications for the evolution of PSII and oxygenic photosynthesis. Progress on the identification of the proteins responsible for Chl *d* synthesis will also be presented.

O036 Phenotypic diversity in the gut: the Salmonella Typhimurium paradigm

Wolf-Dietrich Hardt[1]

[1]*ETH Zurich, Institute of Microbiology, Zurich, Switzerland*

Background: *Salmonella* Typhimurium is a frequent cause of diarrhea. We lack efficient vaccines or therapies for this disease. This might be attributable to particular virulence strategies of this pathogen, i.e. phenotypic diversification. Phenotypic diversification describes the formation of genetically identical subpopulations featuring distinct phenotypes.

Objectives: We suggest that these different subpopulations perform different tasks in overcoming defenses and subverting immune responses. Importantly, such phenotypic diversity may blunt therapeutic interventions failing to target all subpopulations. In a first step, we are deciphering the formation of these subpopulations and study their role in the infection, transmission and pathogen evolution.

Methods: *S*. Typhimurium diarrhea features phenotypic diversity at three different stages of the process. Phenotypic diversity occurs at the intestinal mucus lining, during virulence-factor driven tissue invasion and during the pathogen's interaction with lamina propria phagocytes. In these phagocytes, *S*. Typhimurium forms two different subpopulations with striking differences in growth rates and antibiotic tolerance. About 80% of the phagocyte-lodged pathogen cells grow quickly and promote an early disease onset. This subpopulation is easily killed by cell-penetrating antibiotics like ciprofloxacin. In contrast, about 20% of the tissue-lodged *S*. Typhimurium cells grow slowly and survive antibiotic therapy. We have observed, that this subpopulation can cause relapses after the end of an antibiotic therapy. Moreover, this subpopulation serves as a long-term reservoir for plasmid transmission.

Results: I will discuss how this might promote the spread of antibiotic resistance genes among Enterobacteriaceae. Thus, phenotypic diversity is a fascinating aspect of infection biology with significant practical implications.

O037 Promotion of host cell multinucleation by a Chlamydia trachomatis inclusion membrane protein

Maria Pequito Luís[1], Luís Jaime Mota[1]

[1]*UCIBIO, FCT-NOVA, Caparica, Portugal*

Background: *Chlamydia trachomatis* is a human bacterial pathogen causing genital and ocular infections of major clinical and public health impact. It resides and multiplies exclusively within host cells in a membrane bound vacuole, termed inclusion. *C. trachomatis* delivers several effector proteins into host cells, including Inc proteins, which localize in the inclusion membrane.

Objectives: *C. trachomatis* infection of mammalian cells is known to cause multinucleation, because of an inhibition of host cell cytokinesis. This has been shown to be due in part to the *Chlamydia* protease-like activity factor (CPAF) effector. Because of their privileged position at the bacteria-host cell interface, Inc proteins are likely candidates to also participate in this process, a hypothesis which we tested in this work.

Methods: We constructed a *C. trachomatis incM* gene mutant and used immunofluorescence microscopy to compare the ability of the mutant strain to induce multinucleation in infected cells by comparison to the parental strain and to a complemented strain (*incM* mutant carrying IncM in a plasmid).

Results: We show that cervical epithelial cell lines (HeLa or SiHa) infected by the *incM* mutant strain displayed significantly less multinucleated cells than cells infected by the parental strain. Furthermore, this was reverted when cells were infected by the complemented strain. Thus, IncM likely inhibits host cell cytokinesis. We are currently generating a *cpaf incM C. trachomatis* double mutant to understand if CPAF and IncM participate in the same pathway to promote host cell multinucleation. These results will be presented.

O038 Low ATP consumption increases persister cell formation in Escherichia coli

Marcin Rownicki[1;2;3], Sylvie Manuse[3], Michael Gates[3], Dariusz Bartosik[4], Joanna Trylska[2], Kim Lewis[3]

[1]*University of Warsaw, College of Inter-Faculty Individual Studies in Mathematics and Natural Sciences, Warsaw, Poland*
[2]*University of Warsaw, Centre of New Technologies, Warsaw, Poland*
[3]*Northeastern University, Antimicrobial Discovery Center, Boston, United States*
[4]*University of Warsaw, Department of Biology, Warsaw, Poland*

Background: Most bacteria form persisters, cells that are multidrug tolerant and thus able to survive antibiotic treatment. Recent work from our group showed that ATP depletion is associated with persister formation, which leads to a decrease in the antibiotic target activity. On the other hand, inhibition of translation has been suggested as a universal cause of multidrug tolerance in bacteria.

Objectives: We investigated the relationship between those two persister formation mechanisms. We used chloramphenicol (Cam), a bacterial protein synthesis inhibitor, to induce persistence in *Escherichia coli*

Methods: We evaluated the number of persister cells induced with Cam. Next, we determined the apparent ATP consumption by monitoring the rate of translation and the level of ATP in the cells in bulk population and single-cell level. To monitor translation activity, we measured fluorescent intensity of the GFP. For quantitative measurement of the ATP concentration we used either a luciferase assay or fluorescent ATP indicator “QUEEN”.

Results: We found that pretreatment of *E.coli* with Cam significantly increase the number of persisters. Exposure to Cam had an obvious inhibitory effect on translation and on apparent ATP consumption. Simultaneously, with low level of translation we observed increase in ATP concentration. We conclude that arrested protein synthesis results in a reduction in ATP consumption and an accumulation of intracellular ATP. This leads to increased bacterial persistence. Consistent with our previous findings, changes in ATP levels are linked to low ATP consumption. Therefore, this discovery may provide a more informative readout for persistence than absolute ATP concentration.

O039 Single-cell imaging and characterization of Escherichia coli persister cells to ofloxacin

Frédéric Goormaghtigh[1], Laurence Van Melderen[1]

[1]*Université Libre de Bruxelles, Département de Biologie Moléculaire, Gosselies, Belgium*

Background: Bacterial antibiotic persistence refers to the capacity of small subpopulations within clonal populations to tolerate antibiotics. Persisters are thought to originate from dormant cells presenting reduced metabolic activity, in which antibiotic targets are less active than in dividing cells. Although a few studies managed to directly observe the stochastic switch at the onset of persister cells in wild-type populations of *E. coli* at the single-cell level, those reports were later shown to be problematic. Today, all persisters that were observed at the single-cell level in *E. coli* either originate from high-persistent mutants or were caused by a starvation signal.

Objectives: The aim of the study is to characterize persister cells before, during and after ofloxacin treatment without any a-priori, in wild-type exponentially growing *E. coli*.

Methods: We combined time-lapse microscopy in a microfluidic device with genetically encoded fluorescent reporters and population analyses such as flow cytometry or classical killing assays to characterize persister cells at all stages of their lifecycle.

Results: In contradiction with the prevailent model of slowly or non-growing cells being protected from the antibiotic effect as a result of the inactivity of the antibiotics target, we found that persister cells were actively growing prior to the addition of ofloxacin and did endure DNA damage during exposure to the drug. The unique features of persisters were observed during recovery as they generated long poly-nucleated filaments and underwent a second round of SOS induction, ultimately leading to cell division.

O040 Whole genome DNA methylation (methylome) analysis and role of Dam DNA methyltransferase in the life-cycle of an entomopathogenic bacterium

Payelleville Amaury[1], Legrand Ludovic[2], Lanois Anne[1], Pagès Sylvie[1], Ogier Jean-Claude[1], Blackburn Dana[3], Clarke David[3], Givaudan Alain[1], Julien Brillard[1]

[1]*INRA - Univ. Montpellier*
[2]*INRA - Univ. Toulouse*
[3]*University College Cork*

Background: DNA methylation is an epigenetic mechanism involved in the pathogenicity of several major bacterial pathogens. It can decrease the affinity of some transcriptional regulators to their binding site, leading to sub-populations expressing or not various genes, depending on the DNA methylation state. Dam DNA methyltransferase is widespread in *Gammaproteobacteria* and methylates the adenine of GATC sites.

Objectives: The role of Dam was investigated in *Photorhabdus luminescens* during its symbiosis with a soil nematode and during its pathogenic stage in insects.

Methods: SMRT sequencing (PacBio), which allows identification of the DNA methylation of the whole genome (methylome), RNAseq and phenotypic analysis were performed in a *P. luminescens* strain overexpressing Dam.

Results: Dam overexpression caused a decrease in motility whereas it increased biofilm formation. While symbiosis ability of the Dam overexpressing strain was not significantly different from that of a control strain, the nemato-bacterial complex displayed an impaired pathogenicity in insect, as also observed after direct insect injection of the bacteria alone. Transcriptomic analysis revealed that the observed phenotypes were related to differences at the transcriptional level. More than 99% of the GATC sites of the genome were found methylated and DNA methylation levels did not change over growth kinetics. However, the Dam-overexpressing strain displayed more methylated GATC sites than the control and most of these sites were located in promoter regions. These sites may be involved in the observed differences in phenotypes and gene expression and provide clues to understand the involvement of Dam DNA methylation in *P. luminescens* life-cycle.

O041 Generation of a genetic tool for gauging multiple gene expression at single cell level in Pseudomonas aeruginosa

Marta Mellini[1], Massimiliano Lucidi [2], Gabriella Cincotti[2], Paolo Visca[1], Livia Leoni[1], Giordano Rampioni[1]

[1]*University Roma Tre, Science, Rome,*
[2]*University Roma Tre, Engineering, Rome, Italy*

Background: Single cells of a bacterial population can differ from each other with respect to gene expression, even when genetic and environmental differences between cells are reduced as much as possible. This phenomenon, known as phenotypic heterogeneity, has been described in several bacterial species. However, restricted availability of adequate molecular tools for live monitoring of gene expression at the single cell level has limited our understanding of phenotypic heterogeneity so far.

Objectives: The aim of this project is to investigate heterogeneous gene expression among single cells of bacterial populations, with a special focus on virulence genes in the ESKAPE pathogen *Pseudomonas aeruginosa*.

Methods: In order to investigate phenotypic heterogeneity in *P. aeruginosa*, a genetic tool that allows gauging multiple gene expression at the single cell level has been generated. This tool consists in an integrative promoter-probe vector for transcriptional fusions, carrying three reporter genes coding for three distinct fluorescent proteins.

Results: The newly generated vector has been characterized and validated *via* single cell expression analysis of both inducible and constitutive promoters. Confocal microscopy imaging revealed that the fluorescence signal from each protein can be easily discriminated from fluorescence signals of the other proteins in single cells of a *P. aeruginosa* population. *P. aeruginosa* derivatives carrying vectors for the simultaneous expression profiling of multiple virulence genes have been obtained. Experiments aimed at investigating heterogeneous expression of these genes at the single cell level are in course, both in planktonic and in structured bacterial populations such as biofilms and swarming colonies.

O042 Propidium iodide staining underestimates viability of adherent bacterial cells

Merilin Rosenberg[1;2], Nuno F. Azevedo[3], Angela Ivask[4]

[1]Tallinn University of Technology, Department of Chemistry and Biotechnology, Tallinn, Estonia
[2]National Institute Of Chemical Physics And Biophysics, Laboratory of Environmental Toxicology, Tallinn, Estonia
[3]University of Porto, LEPABE - Laboratory for Process Engineering, Environment, Biotechnology and Energy; Department of Chemical Engineering; Faculty of Engineering, Porto, Portugal
[4]National Institute of Chemical Physics and Biophysics, Laboratory of Environmental Toxicology, Tallinn, Estonia

Background: Intact membrane impermeable DNA-binding stain propidium iodide (PI) is widely used for bacterial viability staining in combination with membrane-permeable counterstains. Extracellular DNA (eDNA) is present in various biofilm matrixes and carries potential to interfere with viability staining results.

Objectives: Our objective was to assess if eDNA can interfere with PI-based viability staining.

Methods: Monolayer aggregates of 24 h *Staphylococcus epidermidis* or *Escherichia coli* on glass in phosphate buffered saline were either stained with PI and Syto 9 *in situ* or harvested via sonication before staining and visualization with epifluorescence (EM) or confocal laser microscopy (CLSM). Fluorescein diacetate (FDA) staining and plate counts were used as controls.

Results: *In situ* stained biofilms consist of 75.69±18.44% to 96.35±5.3% PI-positive red cells for *S. epidermidis* and *E. coli* respectively although 68% the cells of either species are metabolically active when stained with FDA. Higher viability estimates with 19.56±8.93% to 43.50±5.30% PI-positive cells for *S. epidermidis* and *E. coli* respectively were achieved after harvesting adherent cells via sonication probably due to extracellular matrix being partially removed during the process. 82% of harvested *E. coli* and 89% of *S. epidermidis* cells were cultivable. CLSM reveals that this false dead layer of red cells consists of cells that have green interiors under red coating layer confirming extracellular PI signal. In this study we show that PI-based viability staining can significantly overestimate dead cell counts in biofilms and possible impact of eDNA on viability staining outcome must be controlled for to avoid underestimation of biofilm viability.

O043 Linking single cell behaviors to the formation of multicellular patterns in a predatory bacterium

Tâm Mignot[1]

[1]*Laboratoire de Chimie Bactérienne, CNRS- Aix Marseille University, Marseille, France*

Background: A current challenge in developmental biology is to bridge molecular and multicellular scales. This task is especially complex in animals given that the dimension gap spans several orders of magnitude. In this context, multicellular microbes can be especially powerful because their lifecycle rarely exceeds a few days and it can be captured over relatively small surfaces in devices as simple as a petri dish. In addition, these organisms allow sophisticated genetic manipulations and imaging approaches.

Objectives: In our laboratory, we study *Myxococcus xanthus* for its ability to predate and develop collectively over other microbial preys.

Methods: During this presentation, I will present an interdisciplinary approach combining genetics, quantitative imaging and mathematical modeling to decipher how single *Myxococcus* cells direct their movements and cooperate to develop collectively over prey bacteria.

Results: In general, the findings suggest that symmetry breaking and pattern formation arise by short range interactions and propagation from discrete sites in the community.

O044 Phase separation in bacteria revealed by super-resolution microscopies

Marcelo Nollmann[1]

[1]*Centre National de la Recherche Scientifique, Centre de Biochimie Structurale, Montpellier, France*

Background: Many factors in bacteria need to rapidly, precisely and stably localize to specific sub-cellular localizations at specific times of the cell cycle.

Objectives: We investigated the mechanism by which this process may happen by using F-plasmid localization and segregation as a model system.

Methods: We used advanced optical microscopy methods, including super-resolution, time-lapse, and multi-focal microscopies combined to particle-reconstruction methods to discover the mechanism of assembly of the complexe required for plasmid localization and segregation.

Results: We found that a mechanism involving liquid-vapour phase separation is responsible for the formation of the segregation complex. This phase separation requires low-affinity interactions between segregosome proteins as well as specific binding to F-plasmid sequences. Finally, a motor protein is required to maintain segregation complexes in their sub-cellular localizations and to transport them to new places upon plasmid replication.

O045 New insights into bacterial chemoreceptor arrays by electron cryotomography

Ariane Briegel[1], Wen Yang[1], Keith Cassidy[2], Simon Ringgaard[3], John S. Parkinson[4]

[1]*Leiden University, Institute of Biology, Leiden, Netherlands*
[2]*University of Oxford, Biochemistry Department, Oxford, United Kingdom*
[3]*Max Planck Institute, Marburg, Germany*
[4]*University of Utah, Salt Lake City, United States*

Background: Most motile bacteria contain a highly sensitive and adaptable sensory system composed of clusters of chemoreceptors. This chemosensory system is used to detect changes in nutrient concentrations, allows the cells to navigate towards preferential environments and is also involved in host infection by some pathogenic bacteria. While it is one of the best understood signallig systems to date, unraveling structure and function of the bacterial chemotaxis system remains challenging. High-resolution analysis, using methods like X-ray crystallography or nuclear magnetic resonance (NMR), are inherently limited to structural fragments and rely on specimens taken out of their natural environment. Thus, they lack the larger context of the native system.

Objectives: We aim to understand the structure and function of bacterial chemoreceptors in the native context of the array.

Methods: Here, we carried out ECT studies on chemoreceptor signaling arrays. We used subtomogram averaging and Molecular Dynamics Flexible Fitting (MDFF) to study distinctive conformations of receptors in different signaling output states.

Results: Our studies reveal characteristic differences between the chemoreceptor arrays between bacterial species. We further gain insight into the structural differences of receptors in kinase activating and inactivating states in *E.coli*. The receptors exhibited different conformational flexibility in the methylation region between different kinase states, as well as characteristic structural changes in the receptor tips. Our results provide new insights into understanding how conformational dynamics of the receptors modulates the kinase activity.

O046 Building the blocks of a bacterial nano-crossbow

Remi Fronzes[1]

[1]*European Institute of Chemistry and Biology, Pessac, France*

Background: The bacterial Type VI secretion system (T6SS) is one of the key players for microbial competition, and an important virulence factor during bacterial infections. T6SS belong to the broad family of contractile injection systems. All these structures comprise a tail that is composed of an inner tube wrapped by a sheath built under an extended, metastable conformation on an assembly platform, the baseplate. Various signals induce structural rearrangements of the sheath leading to its contraction and to the propulsion of the Hcp-VgrG arrow into the target cell. Assembly of the tail tube/sheath is initiated on the baseplate. In addition to controlling sheath extension, the baseplate also serves to trigger sheath contraction. During T6SS biogenesis, the baseplate docks to a trans-envelope complex composed of TssJ, TssL and TssM.

Objectives: Besides the critical role of the T6SS baseplate and a trans-envelope complexes, we still lack crucial information on their biogenesis and architecture.

Methods: We used hybrid approaches combining cryo-electron microscopy (cryo-EM), biochemical analysis, native mass spectrometry, evolutionary covariance, and molecular modelling to unveil the assembly pathway of the T6SS membrane and baseplate complexes.

Results: We report the detailed structure of the TssKFGE baseplate wedge complex and of the TssJLM complex from the model organism Enteroaggregative *Escherichia coli* (EAEC) Due to the conservation of T6SS wedge and membrane complexes among pathogenic bacteria, the atomic model of the TssKFGE and TssJLM complexes will facilitate the design of new therapeutic molecules to replace or help classical antibiotherapies.

O047 Bacterial transcription regulation: how it works and why it matters

Steve Busby[1]

[1]*University of Birmingham, School of Biosciences, Birmingham, United Kingdom*

Background: Bacteria use a variety of mechanisms to regulate transcription

Objectives: Focussing, on transcript initiation, I will give a brief overview of the different mechanisms that are used.

Methods: Regulatory interactions at bacterial promoters can be studied both *in vitro* and *in vivo*

Results: Recent results from studies at simple and complex *E. coli* promoters will be presented, together with some biotechnology applications.

O048 Identification of the regulon of the extracytoplasmic function sigma factor SigE in Corynebacterium glutamicum

Koichi Toyoda[1], Masayuki Inui[1;2]

[1]Research Institute of Innovative Technology for the Earth (RITE), Molecular Microbiology and Biotechnology Group, Kyoto, Japan
[2]Nara Institute of Science and Technology (NAIST), Graduate School of Biological Sciences, Nara, Japan

Background: Bacteria modify gene expression profile in response to environmental changes to resist and/or adapt to the environmental states. Extracytoplasmic function (ECF) sigma factors play key roles in such expression modification. The ECF sigma factors are bound and inactivated by cognate anti-sigma factors under normal conditions. They are released upon environmental stress, then drive transcription from their specific promoters. For comprehensive understanding of the function of five ECF sigma factors encoded in the genome of Corynebacterium glutamicum, which has been used for the industrial production of amino acids, we have identified the regulons and recognition promoters of SigC, SigD, and SigH. However, the knowledge of the remaining SigE and SigM was limiting.

Objectives: To understand the physiological function of SigE, we determined its regulon and recognition promoter sequence.

Methods: Chromatin immunoprecipitation with microarray (ChIP-chip) analysis was used to identify the genomic regions bound by SigE in vivo. Transcriptome analysis was used for detection of the global expression alteration in gene deletion mutants.

Results: Bioinformatic analysis of the genomic regions detected by ChIP-chip analysis yielded the consensus promoter motif recognized by SigE. This was confirmed by determing the transcriptional start points of genes downstream of the motif. Transcriptome analysis of the anti-sigma factor-encoding cseE deletion mutant defined the SigE regulon. Of the regulon, lppS encodes L,D-transpeptidase, which is required for peptide bond formation of the peptidoglycan. Deletion of sigE or lppS increased lysozyme sensitivity while cseE deletion enhanced lysozyme resistance. Thus, SigE is important for stress resistance.

O049 Overlapping regulons of sigma factors of RNA polymerase in Corynebacterium glutamicum

Miroslav Patek[1], Hana Dostalova[1], Tobias Busche[2], Jiří Holátko[1], Lenka Rucká[1], Václav Štěpánek[1], Ivan Barvík[3], Jan Nešvera[1], Jörn Kalinowski[2]

[1]Institute of Microbiology of the CAS, v. v. i., Prague, Czech Republic, Laboratory of Molecular Genetics of Bacteria, Prague, Czech Republic
[2]Centrum für Biotechnologie, Universität Bielefeld, Bielefeld, Germany, Germany
[3]Institute of Physics, Faculty of Mathematics and Physics, Charles University, Prague, Czech Republic, Czech Republic

Background: *Corynebacterium glutamicum* is a Gram-positive industrial producer of amino acids. Its genome encodes 7 sigma factors of RNA polymerase: primary sigma factor SigA, primary-like SigB and 5 ECF (extracytoplasmic function) sigma factors involved in responses to defects in aerobic respiratory chain (SigC), disturbances of cell wall integrity (SigD), cell surface stress (SigE), heat shock (SigH) or oxidative stress (SigH and SigM).

Objectives: We strive to describe the regulatory network controlled by sigma factors of RNA polymerase in *Corynebacterium glutamicum*. We tried to discover overlapping regulons belonging to two or more sigma factors.

Methods: We combined the genome-wide technique (RNA-sequencing), a single gene analysis (in vitro transcription, in vivo two-plasmid assay for the assignment of sigma factors to genes/promoters and in silico homology modeling of sigma factor-promoter interactions).

Results: We have found that overlaps of sigma regulons (groups of genes transcribed with a particulate sigma factor) are a common strategy how *C. glutamicum* copes with environmental stresses. Many genes are transcribed with the major sigma factor of housekeeping genes, SigA, and with general stress sigma, SigB. A number of SigD-dependent genes were found to be transcribed in a much lower extent with SigH. The upstream regions of these genes also carry in many cases SigA- and/or SigB-dependent promoters. Some of these primarily SigD-dependent genes may be thus alternatively transcribed with as much as 4 sigma factors. Still another regulatory overlap was detected with the SigH and SigE. Finally, expression of some genes is directed alternatively with SigH and SigM.

O050 Testing organizational features linking the genome structure to growth rate in very fast and a very slow growing bacteria

Belén Bordignon[1], Leila Bartrolí[1], Leticia Larotonda[1], Diego Comerci[1], Alfonso Soler-Bistue[1]

[1]*Instituto de Investigaciones Biotecnológicas - Universidad de San Matín, San Martín, Argentina*

Background: The genetic basis of bacterial growth rate (GR) is still unclear. Several features link bacterial genome structure to GR. Comparative genomics show that the number of ribosomal RNA operons (*rrn*) correlate to GR. In parallel, in fast-growing bacteria, the genes encoding for the flow of genetic information- the transcription and translation machineries-are close to the origin of replication (*oriC*). During exponential growth, fast growers perform multi-fork replication. Thus, genes near to *oriC* benefit of higher dosage. Therefore, such positional bias can be a strategy to maximize RNAP expression.

Objectives: We aim at experimentally testing these correlations in slow and fast-growing bacteria.

Methods: We used Bradyrhizobia as a model for slow-growing bacteria. *Vibrio cholerae* is a fast GR whose genome can be widely modified by natural transformation coupled to recombineering techniques based on lambda phage recombination sites. This allowed altering the genomic location of S10-spc-α (S10) and the *rpoBC* locus which harbor most of ribosomal proteins and RNA polymerase genes respectively.

Results: Growth curves testing different isolates showed that Bradyrhizobia bearing 2 rrn grew faster than those bearing a single operon, independently of the culture media tested. In *V. cholerae*, the relocation of S10 or *rpoBC* loci far form *oriC* led to lower GR. Close relocation displayed no phenotype indicating that relocation process per se was not detrimental. The relocation of S10 caused a stronger effect. These physiological alterations are probably due to differences in gene dosage that occurs during the exponential phase due to overlapping replication rounds.

O051 Macromolecular crowding links ribosomal protein gene genomic position to growth rate in Vibrio cholerae

Alfonso Soler-Bistue[1,2], Sebastian Alguilar Pierlé[2], Marc García-Garcera[2], Marie-Eve Val[2], Diego Comerci[1], Eduardo P. Rocha[2], Didier Mazel[2]

[1]*Instituto de Investigaciones Biotecnológicas - Universidad de San Matín, San Martín, Argentina*
[2]*Institut Pasteur, Génomes et Génétique, Paris, France*

Background: Ribosomal protein (RP) genes locate near the replication origin (*oriC*) in fast-growing bacteria. Thus, higher RP dosage during exponential phase due to overlapping replication rounds would optimize translation levels. We built a set of strains where *S10-spc-α* locus (S10), coding most of RP, was relocated to alternative genomic positions. S10 relative distance to the *oriC* tightly correlates to reduction of its dosage and growth rate.

Objectives: Uncover mechanisms linking S10 dosage to bacterial physiology.

Methods: We studied strains were only S10 location was altered. We estimated protein production. We deep sequences their DNA and RNA. Flourescence Recovery After Photobleaching (FRAP) allowed cytoplasm fluidity assessment.

Results: S10 location did not affect protein production at the population level. At the single-cell level, there was less reporter synthesys when S10 was far from *oriC*. RNA and DNA deep-sequencing revealed genome-wide alterations transcription and replication dynamics. The number of differentially expressed genes and replication dynamics varied as a function of S10-*oriC* distance. Transcriptionally altered genes were implicated in amino acid metabolism, transport and protein folding. Since RP constitute a significant propotion of cell mass we hypothesized that lower S10 expression could reduce the cytoplasm macromolecular crowding, globally impairing cell physiology. We gathered evidence supporting this idea. FRAP experiments showed higher cytoplasm fluidity when S10 dosage is low. Differences in growth and replication dynamics in the most affected mutans were alleviated in a hyperosmotic context. These experiments suggest that RP location besides optimazing protein biosynthesis, provides the ideal macromolecular crowding conditions.

O052 Novel insights into the CbrAB expression network in Pseudomonas putida

Ines Canosa[1], Rocío Barroso[1], Elizabet Monteagudo-CAscales[1], Sofía M. GArcía-Mauriño[1], Laura Tomás-Gallardo[1], Eloísa Andújar[2], Mónica Pérez-Alegre[2], Eduardo Santero[1]

[1]Universidad Pablo de Olavide, CABD/CSIC/JA, Molecular Biology and Biochemistry, Sevilla, Spain
[2] CABIMER/ CSIC/ Univ. de Sevilla/ Univ. Pablo de Olavide/ JA

Background: The ability to recognize and convert external environmental stimuli into appropriate physiological responses is of fundamental importance for all organisms. In Pseudomonads, the two-component regulatory system CbrAB is responsible to the adaptation to carbon limitation. Global analysis of the regulatory element CbrB in *P. putida* shows that it controls a collection of σ^N-dependent genes involved in the assimilation of several amino acids but also in a number of other physiological functions, such as chemotaxis or stress tolerance. Conversely, very little is known about the signal that triggers the Cbr response, which is channeled through the histidine kinase CbrA. CbrA represents a new family of sensor HKs as its structure suggests it may link signalling to transport of a molecule. Its N terminus contains a 13 TM domain with similarity to the sodium-proline symporter PutP from *E. coli*, which is connected to the C terminal catalytic HK domain which could regulate transport through the sodium/solute symporter domain.

Objectives: Describe the CbrB regulon for a consensus CbrB-binding sequence description and determine its contribution of in the transcriptional activation. Also , explore the regulation of CbrA expression and the implication of the TM, PAS and HK domains in the signal transduction.

Methods: ChIP-seq analysis and point mutagenesis to the DNA sequences necessary for its binding and transcriptional activation. Also, we explore the control of *cbrA* expression and constructed truncated versions of CbrA to follow the signal transduction.

Results: Altogether, we show an extensive view on the molecular mechanism of CbrAB activation.

O053 Regulation of tert-butyl hydroperoxide resistance by chromosomal OhrR in Acinetobacter baumannii ATCC19606

Guang-Huey Lin[1], Shih-Jie Chen[2]

[1]Tzu Chi University, Master Program in Microbiology and Immunology, Hualien, Taiwan
[1]Tzu Chi University, Master Program in Microbiology and Immunology, Hualien, Taiwan

Background: OhrR is a transcriptional regulator of MarR family. In this study, we show that *Acinetobacter baumannii* ATCC 19606 harbors two sets of *ohrR-ohr* genes, respectively encoded in chromosomal DNA and a pMAC plasmid.

Objectives: Study the function of chromosomal OhrR in *A. baumannii* ATCC19606

Methods: Marker-less mutation were conducted to construct each of *ohrR-ohr* mutants for functional ayalysis. By using electrophoretic mobility shift assay (EMSA), chromosomal OhrR regulated genes were determined.

Results: We found no significant difference in organic hydroperoxide (OHP) resistance between strains with or without pMAC. However, a disk diffusion assay conducted by exposing wild-type, *ΔohrR* or *Δohr* single mutants, or *ΔohrRΔohr* double mutants to *tert*-butyl hydroperoxide (TBHP) found that the *ohrR-ohr* genes may be able to complement the function of their chromosomal counterparts. In a survival assay conducted with *Galleria mellonella* larvae, these mutants demonstrated almost complete loss of virulence. By EMSA, we found that OhrR was able to bind to the promoter regions of both chromosomal and pMAC *ohr* genes, but with varying affinity. A gain-of-function assay conducted in *Escherichia coli* showed that OhrR was not only capable of suppressing transformed *ohr* genes, but may also repress endogenous enzymes. Taken together, our findings suggest that chromosomal *ohrR-ohr* genes act as the major system in protecting *A. baumannii* ATCC 19606 from OHP stresses, but the *ohrR-ohr* genes on pMAC can provide a supplementary protective effect, and the interaction between these genes may affect other aspects of bacterial viability and virulence.

O054 RcsG, a connector of PTS to TCS in Salmonella enterica

Akinori Kato[1]

[1]*Kindai University, Department of Advanced Bioscience, Nara, Japan*

Background: Connectors are small proteins that mediate integration of signal(s) of a two-component system (TCS) into outcome(s) of a TCS in bacteria. For example, PmrD transfers the signal of the PhoP/PhoQ system into the PmrA/PmrB system, thus enabling *Salmonella enterica* resistant to polymixin B in response to not only Fe^{3+} but also to low Mg^{2+}; the PhoP-activated small protein MgrB inhibits PhoQ activities, closing the feedback loop.

Objectives: The facts that emergence of resistance to the last resort antibiotic colistin is largely due to mutations in the regulatory network linked by the connectors, PmrD and MgrB, in *S. enterica* and closely related Gram-negative pathogens draw attention on investigating novel connectors.

Methods: Connectors do not share significant homology in general. On one hand, this could allow specific interaction between a connecter and its target TCS. On the other hand, it is difficult to predict them from bacterial genomes. To uncover novel connectors involved in antibiotic resistance and virulence regulation genetically in *S. enterica*, we have created and utilized small genes library.

Results: Here, we report a novel connector RcsG that links phosphotransferase system (PTS) and the TCS RcsC/RcsD/RcsB. *rcsG* was found as a clone that activated the RcsCDB-regulated *wza-lac* fusion and a small gene, part of a cluster including PTS related genes on the *Salmonella* geneome. In a spontaneous mutant background, *rcsG* was expressed in *ptsI*- and *ptsH*-dependent manners. RcsG interacted with RcsD in the bacterial two-hybrid system. We will discuss further the mechanism of action of RcsG.

O055 Novel two-component system-like elements reveal functional domains associated with restriction-modification systems in bacteria

Daniel Bellieny-Rabelo[1], Willem Pretorius[1], Divine Shyntum[1], Lucy Moleleki[1;2]

[1]Faculty of Natural and Agricultural Sciences, Department of Biochemistry, Genetics and Microbiology, Pretoria, South Africa
[2]FABI, Pretoria, South Africa

Background: A dominant cellular signaling archetype in nature is comprised by a receptor/transmitter protein paired with a ligand-binding regulator to be affected downstream, collectively known as two-component systems (TCS). In bacteria, histidine-kinases (HKs) are the most common transmitter proteins in TCS. HKs commonly associate with intracellular response regulators (RR), which transduce signals and generate responses through diverse strategies, which may range from binding DNA or other protein to enzymatic catalysis.

Objectives: In this study, we utilize sequence analysis to identify and characterize two novel protein domains which exhibit characteristic structural features found respectively in HKs and RRs, in a wide range of bacteria. In addition, we aim to further expand functional and regulatory information on members of the new domain families by conducting extensive gene-neighborhood analyses.

Methods: Multiple sequence alignments and secondary structure predictions were conducted to allow comparisons between the new domains and canonical HK and RR domains. Newly consolidated hidden Markov model based profiles were used to expand detection of protein sequences exhibiting the novel domains with high sensitivity in public databases.

Results: Results revealed 94% linkage between the members of the two predicted HK and RR domains across 519 bacterial strains. In addition to the solid structural evidence, this prominent linkage suggests that members of these two families might comprise a novel TCS. Furthermore, the strong presence of restriction-modification systems in the same genomic neighborhoods of these TCS-like modules obsereved in a large phyletic spectrum may suggest a role in regulation of protection against foreign DNA.

O056 Antibiotics in sewage: should we be worried?

Mike Dempsey[1]

[1]*Advanced Bioprocess Development Ltd, Salford, United Kingdom*

Background: Because patients taking antibiotics generally excrete a majority of the given dose, much of this ends up in the aquatic environment, either *via* discharge of treated sewage or direclty in countries without sewage collection or treatment. Given the problem of antibiotic resistance, minimising release of antibiotics to the natural environment is of importance. Therefore, it is important to understand the fate of excreted antibiotics during passage through sewage treatment processes, so that improved removal can be achieved, either through optimised process operation or the development of new process technologies.

Objectives: I will outline the main processes involved in sewage treatment, to provide essential background information for a more complete understanding of the subsequent talks. In particular, I will focus on the biological processes that might be involved.

Methods: My talk will not include experimental results but will, instead, focus on methods of sewage treatment. For example, trickling filters, the activated sludge process, sequencing batch reactors, and more modern biofilm-based processes.

Results: Although there are no experimental results to present, different process technologies will be evaluated in terms of either their actual or potential removal efficiency.

O057 Antibiotic removal during biological wastewater treatment - Reactor design and modelling of biofilm systems

Benedek Plosz[1]

[1]*University of Bath, Chemical Engineering, Bath, United Kingdom*

Background: The concept of Moving Bed Biofilm Reactors is recognized as a cost effective solution for upgrading wastewater treatment plants to meet e.g., EU limit of carbon and nitrogen in the effluent. Compared to activated sludge systems, increased micropollutant removal efficiency was reported for numerous pharmaceuticals in biofilm systems.

Objectives: A review of recent advances in antibiotic micropollutant (AMP) removal from wastewater is presented. The main focal areas include (i) enhanced removal in biofilm reactor systems; (ii) modelling factors influencing AMP removal in biofilm; and (iii) relevance to water resource recovery for agricultural reuse.

Methods: Laboratory-scale biofilm reactors systems (heterotrophic denitrifying and autotrophic nitrifying conditions. Molecular methods for microbial community composition: Illumina sequencing, quantitative PCR and FisH. One-dimensional advection-diffusion simulation models using Aquasim and MatLab.

Results: Regarding potential design solutions to enhance biogeochemical AMP, biofilm reactor staging was assessed as a means to engineer microbial community composition to enhance AMP removal through heterotrophic denitrification. Additionally, the impact of external carbon dose on AMP removal was assessed using methanol and ethanol in post-denitrifying biofilm systems. Moreover, an innovative biofilm carrier with the propensity of maintaining defined biofilm thickness was evaluated as a means to increase AMP removal in moving bed nitrifying biofilm reactors.

Additionally, advances in modelling factors influencing AMP removal in biofilm in terms of diffusion and sorption of AMPs as well as the impact of growth substrate availability on APM removal is presented. Finally, the significance of biogeochemical AMP removal on sustainable wastewater resource recovery technology is evaluated.

O058 Understanding the sorption and biotransformation of antibiotics in innovative biological wastewater treatment technologies

Teresa Alvarino[1,2], Francisco Omil[2], Juan Lema[2], Sonia Suárez[2]

[1]*Cetaqua Galicia, Santiago de Compostela, Spain*
[2]*Universidade De Santiago De Compostela, Chemical Engineering, Santiago de Compostela, Spain*

Background: In the last years, new wastewater treatment technologies based on the combination of different redox conditions and biomass conformation have been developed.

Objectives: This research is focussed on understanding the sorption and biotransformation of antibiotics in these innovative technologies due to the information about this topic is scarce.

Methods: A comparative analysis of different biological wastewater treatments were carried out to understand which operational parameters influence the sorption and biotransformation of the antibiotics. Factors such as biomass conformation and particle size, upward velocity applied or the addition of adsorbents have been considered.

Results: - The sorption onto the sludge of antibiotics is affected by the biomass conformation and the particle size. In the case of the granular biomass, the sorption equilibrium is not quickly achieved and is affected by several parameters, such as the hydraulic or the sludge retention time.

- In membrane bioreactors, the physicochemical characteristics of the membrane determine its sorption capacity. For instance, hydrophobic membranes can absorb lipophilic compounds, whereas charged membranes are able to retain antibiotics by electrostatic interactions.

- The cometabolic removal of antibiotics depends on several parameters, such as the biomass concentration or the biomass primary metabolic activity.

- The antibiotics biotransformation depends on the applied redox potential. The chemical structure of the antibiotics determines if its is prone to be removed by positive or negative redox conditions.

- The integration of different redox potentials in hybrid biological configurations constitutes a good alternative to improve the removal efficiencies of antibiotics.

O059 Fate and effects of cephalosporin antibiotics in the aquatic environment

Alyson Rogério Ribeiro[1;2]

[1]*Instrumental Analytical Chemistry and Centre of Water and Environment Research (ZWU), University of Duisburg-Essen, Essen, Germany*
[2]*Institute of Chemistry, Department of Analytical Chemistry, University of Campinas, Campinas, Brazil*

Background: Human and veterinary cephalosporins can reach the environment due to their widespread consumption, intermittent and diffuse discharge rate. Particular attention is given to this antibiotic group due to their broad spectrum of activity and possible deleterious effects in non-target organisms as well as pressure in antimicrobial resistance acquirement, which may be caused by the parental drugs, metabolites and bioactive byproducts. Furthermore, fundamental abiotic degradation processes such as hydrolysis mechanisms and rates are scarce for several cephalosporins. Likewise, the behaviour of such compounds during water disinfection strategies (i.e. photolysis) and the implications concerning ecotoxicological effects are still unknown.

Objectives: This work aimed at the investigation of the fate and effects of cephalosporin antibiotics in the aquatic environment.

Methods: Physicochemical properties, consumption, occurrence in aqueous matrices, available ecotoxicity data and technical strategies employed for the degradation of compounds within this group were critically discussed.

Results: Classical biological treatment systems cannot provide complete removal of cephalosporins, therefore several technologies have been employed for the removal of these compounds from aquatic matrices. In this regard, photolysis and mass transfer processes were the most investigated ones. Cefalexin, cefradine, cefotaxime and cefazolin possess respectively the highest occurrence score in the aquatic environment. Besides the scarcity of data concerning this group, topics were identified where further investigations are necessary, as: ecotoxicological assessment of parental and transformed compounds, especially using soil organisms, cyanobacteria and biofilms; abiotic degradation rates (i.e. hydrolysis, photolysis); analysis of biologic inactivation and inclusion of metabolites and transformation products in surface water surveillance.

O060 Conserved eukaryotic polarity proteins direct the spatial organisation of multiple cellular processes, including directional growth, in the fungus, C. albicans

Alexandra Brand[1], Tina Bedekovic[1], Mariana Almeida[1], Angela Lopez Garcia[1]

[1]*University of Aberdeen, MRC Centre for Medical Mycology, Aberdeen, United Kingdom*

Background: The fungus, *Candida albicans*, grows as a commensal yeast in humans. In some, it can cause irritating mucosal infections but, in severely immunocompromised patients, it can escape into the bloodstream to cause life-threatening systemic disease. A primary virulence trait of *C. albicans* is the morphological switch to hyphal filaments that penetrate barrier-cell layers and disrupt underlying tissue, causing sepsis and organ failure.

Objectives: Our aim is to understand how *C. albicans* hyphae regulate their direction of growth in response to physical aspects of the host environment. Our objectives are to define the sensing and signalling pathways that regulate the positioning of cell polarity mechinery during consitutively polarised hyphal growth.

Methods: We have developed a microfabricated, live-cell imaging system in which we have quantified real-time directional responses and growth behaviours in *C. albicans*. By generating gene deletion mutants and strains expressing GFP-tagged proteins, we have observed the temporal and spatial reorganisation of polarity and cell-growth proteins as the hyphal tip encounters various topographical cues.

Results: We have quantified the force applied by *C. albicans* hyphae as 8.7 µN, but this is dependent on carbon source. The key regulators of directional responses, such as Cdc42, Rap1/Rsr1 and Paxillin, appear to be conserved across eukaryotic cells, but contact-dependent responses are an emergent property of fungus-specific biology and lifestyle. Using live-cell imaging and proteomics, we find that polarity proteins direct multiple processes that require the spatial organisation of cellular components, including nuclear division, nuclear migration, vacuole fusion and septum formation.

O061 Transcriptomic analysis of A fumigatus during natural noninvasive sinonasal infections in canine patients

Ivan Valdes[1], Han Wösten[1], Hans de Cock[1]

[1]*Utrecht University, Utrecht, Netherlands*

Background: Sino-nasal aspergillosis (SNA) is a chronic erosive non-invasive fungal sinusitis characterized by destruction of nasal- and bone tissue. Fungal plaques in dogs are white indicated no asexual development and have a high pH. Each fungal plaque was composed of one particular fungal genotype but individual isolates from each plaque had a high phenotypic variability suggesting in-host adaptation.

Objectives: Transcriptomic profiling of fungi in vivo during natural infections can provide new insights in host-pathogen interactions that might be missed in model infection systems.

Methods: RNA was isolated from fungal plaques and sequenced. After cleaning and trimming of reads using Fastx-toolkit Kallisto was used for transcript quantification (TPM) with A. fumigatus Af293 (AspGD) as reference. Shared genes in all fungal plaques had either a low, medium or highly stable expression. Enrichment analysis and categorization of the transcriptome was performed using published lists of genes involved in stress, reproduction and virulence.

Results: Fungal transcriptomes shared 8029 genes and 13% had a highly stable expression level including genes involved in general metabolism and stress response. Fungal expression profiles were most similar when derived from the same canine patient, despite the fact that these fungi had different genotypes. Only 6% of the shared genes showed high variability, including genes involved in secondary metabolite expression and defense related and resistance proteins like MFS and ABC transporters. Our results provide evidence for the absence of asexual reproduction and high pH in the sinus.

O062 Rme1 regulates chlamydosporulation in Candida albicans

Iryna Denega[1;2], Arturo Hernández-Cervantes[1], Sadri Znaidi[1;3;4], Lasse van Wiljick[1], Christophe D'Enfert[1], Sophie Bachellier-Bassi[1]

[1]*Institut Pasteur, INRA, Unité Biologie et Pathogénicité Fongiques, Paris, France*
[2]*Université Paris Diderot, Sorbonne Paris Cité, Cellule Pasteur, Paris, France*
[3]*Institut Pasteur de Tunis, Laboratoire de Microbiologie Moléculaire, Vaccinologie et Développement Biotechnologique, Tunis-Belvédère, Tunisia*
[4]*University of Tunis-El Manar, Tunis, Tunisia*

Background: The opportunistic human fungal pathogen *C. albicans* is able to adopt a wide range of morphological forms in response to environmental changes. For instance, *C. albicans* differentiates into chlamydospores when incubated under nutrient-poor conditions in darkness. Chalmydospores are large, spherical, thick-walled structures filled with lipid inclusions, often growing from filaments. To date, the functional role of chlamydospores, as well as pathways controlling their formation, remain elusive. Our team have demonstrated the role of the *C. albicans* transcription factor (TF) Rme1, an ortholog of *Saccharomyces cerevisiae* regulator of meiosis, in regulating this process.

Objectives: To identify transcriptional networks governing the morphogenetic program of chlamydosporulation in *C. albicans*.

Methods: *C. albicans* reference strains SC5314, BWP17 and their derivatives were used in this study together with clinical isolate CEC2018. Chlamydosporulation was performed on PCB (potato-carrot-bile) medium (Pavlatou *et al*., 1961). ChIP and transcriptomic analyses were used to identify targets of Rme1 binding.

Results: ChIP experiments suggested a role of Rme1 in chlamydosporulation. In agreement, a *rme1* knock-out (KO) strain was deficient for chlamydospore formation, while overexpression (OE) of *RME1* increased their number. Further, OE of *RME1* bypassed the requirement of Hog1 and Efg1, two regulators essential for chlamydosporulation. We also showed that the Sfl1 and Ndt80 TFs regulated Rme1 antagonistically, with opposite effects on chlamydospore formation. Screening of a collection of TF KO strains has now identified additional regulators of chlamydosporulation. Data will be presented that evaluate the link of these TFs with Rme1 and further unveil the regulatory network governing chlamydosporulation.

O063 Candida albicans pH-regulated antigen 1 (Pra1), expression and interaction

Mohammad Alsarraf[1], Duncan Wilson[1]

[1]*University of Aberdeen, Institute of Medical Sciences, Aberdeen, United Kingdom*

Background: Candida albicans Pra1 is secreted, binds zinc, and reassociates with the fungal cell via a syntenically encoded plasma membrane transporter, Zrt1. This zincophore system contributes to *C. albicans* pathogenicity by scavenging micronutrient. However, little is known about the regulation of Pra1 expression and interaction between components of zincophore system.

Objectives:

1. To examine the effect of extracellular zinc bioavailability and ambient pH on the expression of *PRA1*.
2. To understand dynamic interaction between Pra1 and Zrt1.

Methods: To explore expression of Pra1, mCherry-tagged version of Pra1 was constructed, the strain was grown in with different pH or zinc concentrations, and next, fluorescence was measured. To understand interaction of zincophore components, mCherry-tagged version of PRA1 was complemented into *zrt1*Δ/ *pra1*Δ double mutant lacking native Zrt1 and *pra1*Δ strains; also, C-terminus truncated version of Pra1 was generated, and difference in fluorescent was tested using microscopically and microplate reader.

Results: The expression of Pra1 was highly regulated by the ambient pH, mCherry fluorescence for strains grown in Limited Zinc Media pH 7.5 was highest; indeed, our data showed that expression of Pra1 is regulated by micromolar concentration of zinc. In addition, data showed cell fluorescent of strain with native Zrt1 was three times higher when compared with a strain lacking Zrt1 receptor or lacking C-terminus of Pra1, suggesting the importance of Zrt1 as a receptor for reassociation of Pra1 to Candida cells, and C-terminus of Pra1 is crucial for interaction with Zrt1 receptor.

O064 Ochratoxin A and citrinin production and migration in mold contaminated cheese

Monika Coton[1], Arnaud Auffret[1], Elisabeth Poirier[1], Stella Debaets[1], Emmanuel Coton[1], Philippe Dantigny[1]

[1]*Université de Bretagne Occidentale, LUBEM EA3882, Plouzané, France*

Background: *Penicillium verrucosum* is a fungal species contaminating foods, such as cheeses, that can lead to significant losses. *P. verrucosum* can produce toxic extrolites, including ochratoxin A (OTA) and citrinin (CIT), which can cause serious physiological effect in humans.

Objectives: The objective of this study was to develop an experimental plan to evaluate the migration of these two mycotoxins in *P. verrucosum* contaminated Comté cheese.

Methods: Multiple strains were compared for growth then mycotoxin production on cheese. A strong OTA/CIT-producing strain was then selected and a spore suspension was inoculated onto cubes of Comté cheese and incubated at 8°C (for 42 days) or 20°C (for 28 days) to mimic typical storage at the consumer level. Fungal growth was monitored and sampling for OTA/CIT determination was carried out at regular time intervals. Mycotoxins were then extracted and quantified using LC-QTOF from 2 mm thick cheese slices cut out from 4 cm^3 cheese cubes as follows: 0-2, 2-4, 4-6 and 6-8 mm.

Results: Using this methodology, CIT and OTA production and migration was monitored in Comté cheese for both storage temperatures. A clear shift in secondary metabolite biosynthesis was observed for samples stored at 8°C as CIT production was highest at 14d, then decreased, while OTA production started at 28d. For samples stored at 20°C, simultaneous production and migration of both metabolites was observed from 7d on. The obtained data can be used for risk assessment and we have proposed recommendations for consumers in the case of mold contaminated cheese.

O065 The involvement of the UPR pathway in adaptive responses to antifungal toxicity in Trichophyton rubrum

Tamires Aparecida Bitencourt[1], Pablo Rodrigo Sanches[1], Vanderci M. Oliveira[1], Antonio Rossi[1], Nilce Maria Martinez-Rossi[1]

[1]*University of Sao Paulo, Ribeirao Preto Medical School, Department of Genetics, Ribeirao Preto, Brazil*

Background: *Trichophyton rubrum* is the most common agent of dermatophytosis worldwide. The endoplasmatic reticulum (ER) is the gateway for the secretion pathway, responsible for post-translation modifications, protein folding, and also participates in lipid synthesis. It responds for a myriad of stimuli, both in and extra cellularly. Overwhelming the ER folding capacity of protein secretion threaten homeostasis. To cope with this stress the unfolding protein response pathway (UPR) is triggered. In yeast and other fungi, the accumulation of unfolding proteins is sensed by transmembrane protein IRE1, which in turn cleaves an intron in downstream mRNA HAC1, allowing it translation, followed by an up-regulation of genes required under stress.

Objectives: The purpose of this work was to characterize molecular aspects of UPR in *T. rubrum* after its exposure to antifungal compounds.

Methods: RT-PCR and qRT-PCR assessed the gene modulation of *ire1* and *hac1* after *T. rubrum* exposure to antifungals. Besides we analyzed the cleavage site of *hac1* from *T. rubrum* using bioinformatics tools.

Results: The HAC1 activation occurs in the presence of both griseofulvin and terbinafine. Furthermore, we showed an up-regulation of *ire-1* and *hac1* after exposure of *T. rubrum* to terbinafine. The *hac1* splicing in *T. rubrum* removes 20 nt, corresponding to part of exon-2 and intron-2. With a change in the open reading frame, a new DNA binding site arises. Thus, these results suggest that UPR is switched on by terbinafine exposure as an adaptive response to mitigate the lipid perturbation inside the ER. Financial support: FAPESP, CAPES, CNPq, FAEPA.

O066 #ScienceSolstice: citizen science meets mycology

Jennifer Shelton[1], Thomas Sewell[1], Johanna Rhodes[1], Andrew Singer [2], Marta Blangiardo [3]

[1]*Imperial College London, Department of Infectious Disease Epidemiology, School of Public Health, United Kingdom*
[2]*Centre for Ecology and Hydrology (CEH), Wallingford, Oxfordshire, United Kingdom*
[3]*Imperial College London, Department of Epidemiology and Biostatistics, School of Public Health, United Kingdom*

Background: *Aspergillus fumigatus* is a facultative human pathogen and ubiquitous in the environment—responsible for decaying plant matter. *A. fumigatus* has acquired drug-resistance in the last 20 years, with evidence points to the use of azole-fungicides in agriculture as a driver of the resistance seen in the clinic.

Objective: #ScienceSolstice is a citizen science project aimed at collecting *A. fumigatus* spores from across the UK using a novel, passive air sampling methodology. A. fumigatus spores on samplers are quantified and assayed for azole-fungicide resistance. Potential drivers of azole resistance, i.e., composting sites and agricultural crops sprayed with azoles, are to be statistically analysed for geospatial correlations.

Method: Air samplers left on windowsills for 8 hr were deployed by 485 UK participants and 66 global participants and sent back to the lab for growth and resistance testing.

Results: The initial collection on 21st June 2018 (summer solstice) attracted 551 participants who collectively returned a total of 864 air samples, which grew 1,431 *A. fumigatus* colonies. The second collection on 24th September 2018 (autumn equinox) attracted 306 participants who returned 490 air samples, which grew *A. fumigatus* colonies. Analysis of winter solstice air samples is currently underway and the next collection round will be in March 2019. Resistance testing is on-going against tebuconazole- the third most used azole in agricultural fungicides. Early evidence reveals pockets of highly-resistant spores across the UK. Future work will reveal the proximity of known drivers of azole resistance.

O067 Modelling human skin infections using human skin explants

Dora Corzo Leon [1], Carol Munro[1], Donna MacCallum[1]

[1]*University of Aberdeen, Aberdeen Fungal Group, Aberdeen, United Kingdom*

Background: Around one quarter of the world's population suffer from fungal skin infections at any one time, with the majority of infections caused by dermatophytes and *Malasezzia* species. Fungal skin infections are difficult to model in animals as many dermatophyte infections progress, and are resolved, differently in animals. This makes it difficult to study fungal-host interactions and responses to therapy for these pathogens.

Objectives: In order to more accurately model human skin fungal infections we chose to develop an infection model using human skin explants.

Methods: Excess human skin from surgical procedures was obtained commercially and had the adipose layer removed. Skin was incubated in tissue culture medium, maintaining a liquid-air interface, so that the skin surface remained dry and exposed to air. Fungi were inoculated directly on to undamaged skin or onto skin which was damaged by a needle. Infection was monitored by microscopy and host responses by qRT-PCR, microscopy and proteomics.

Results: We show that human skin explants remain viable for up to two weeks. We demonstrate that some fungi, such as *Trichophyton rubrum* (athletes foot), are capable of initiating infection without prior damage, where others, such as *Fusarium*, require damage to establish an infection. Using microscopy we show that infection induces apotosis in skin and that the skin responds to infection through expression of antimicrobial peptides and cytokines/chemokines, with different responses seen for different fungi. Finally, we show that our model can be used to model effective antifungal therapy.

O068 Indoormyco: DNA based profiling of indoor mycobiomes

Eva Lena Estensmo[1], Inger Skrede[1], Pedro Maria Martin-Sanchez[1], Torbjørn Rognes[2], Ingeborg Bjorvand Engh[3], Johan Mattsson[3], Håvard Kauserud[1]

[1]*University of Oslo, Department of biosciences, Oslo, Norway*
[2]*University of Oslo, Department of informatics, Norway*
[3]*Mycoteam AS, Norway*

Background: Health risks connected to the indoor microbiome are mostly associated with poor indoor air quality. Wherever excess moisture is available, fungi and other microorganisms can start to grow and act as a source of indoor pollutants. This causes poor indoor air quality that is associated with adverse health effects, such as allergies, asthma and other respiratory symptoms. In other parts of the world, several studies have been performed trying to identify the main determinants and important factors influencing the indoor mycobiome. These factors commonly include building type, geography, ventilation and outdoor air influence amongst others.

Objectives: Therefore, the overarching aim of the IndoorMyco project is to improve the knowledge about the indoor mycobiome in Northern Europe by using DNA high throughput sequencing (HTS) analyses. By analyses of dust samples, the indoor mycobiomes will be characterized at different spatiotemporal scales; within buildings, across buildings at larger geographical scales, as well as throughout different seasons.

Methods: Sampling has been performed within Norwegian kindergartens throughout a year by biweekly sampling at four different floors and by large scale sampling of 128 kindergartens throughout Norway. Dust has been collected from specific areas on identical glass plates or on door shelves by using floq swabs and tape. Real-time PCR has been performed covering the 20 most common fungal species in the indoor air, including Aspergillus, Penicillium, Alternaria and Chaetomium amongst others.

Results: Compositional differences in the indoor mycobiomes will be coupled to local and regional environmental variation and building characteristics through multivariate analyses.

O069 Regulation of biofilm formation in Salmonella typhimurium and Escherichia coli

Ute Römling[1]

[1]*Karolinska Institutet, Department of Microbiology, Tumor and Cell Biology, Stockholm, Sweden*

Background: Biofilm formation is a fundamental life style of microorganisms. The transition from motility to sessility leading to biofilm formation is regulated by the ubiquitous second messenger cyclic di-GMP with *Salmonella typhimurium* and *Escherichia coli* being no exception.

Objectives: To unravel the molecular mechanisms of regulation of biofilm formation

Methods: A spectrum of molecular microbiology approaches

Results: In these two species, cyclic di-GMP positively regulates the expression of the orphan transcriptional regulator *csgD*, a major hub of biofilm formation. Expression of *csgD* in *S. typhimurium* is dependent on many environmental conditions and requires the catalytic activity of at least four diguanylate cyclases, four cyclic di-GMP specific phosphodiesterases and the protein scaffold of the GGDEF-EAL domain protein YciR. In a downstream pathway, CsgD activates production of the extracellular matrix component cellulose through the diguanylate cyclase AdrA, which synthesizes the cyclic di-GMP for post-transcriptional regulation of cellulose. In addition, cellulose production is regulated by the accessory protein BcsG. Stable production of the cellulose synthase BcsA requires the membrane-part of the alkaline phosphatase superfamily member BcsG, while its phospholipid headgroup transferase activity is required for production of the cellulose macromolecule. Horizontal transfer of novel signaling systems such as the cyclic GAMP signaling system might add another level of complexity to the already sophisticated regulation of biofilm formation.

O070 The BDSF quorum sensing signal controls polysaccharide production in Burkholderia cenocepacia via modulating the intracellular c-di-GMP level

Elisabeth Steiner[1], Anja Richter[2], Nadine Schmid[1], Tim Tolker-Nielsen[2], Leo Eberl[1]

[1]*University of Zürich, Department of Plant and Microbial Biology, Zürich, Switzerland*
[2]*University of Copenhagen, Department of International Health, Immunology and Microbiology, Copenhagen, Denmark*

Background: The secondary messenger c-di-GMP is a key factor controlling the transition between motile and sessile lifestyles, with high c-di-GMP levels being indicative of the biofilm mode of growth. In addition to its role in biofilm formation, c-di-GMP is also involved in the regulation of many cellular functions, often in connection with virulence. The cystic fibrosis isolate *Burkholderia cenocepacia* H111 uses three chemically distinct signal molecules for controlling gene expression in a cell density-dependent manner. One of these is cis-2-dodecenoic acid (BDSF) which, upon binding to its cognate receptor RpfR, lowers the intracellular c-di-GMP level.

Objectives: In *B. cenocepacia* H111 c-di-GMP controls the production of the exopolysaccharide Bep, which is essential for a wrinkled macrocolony morphology on nutrient agar plates and pellicle formation in standing cultures. Given that an rpfR mutant shows a wrinkled colony morphology we investigated the role of this signalling system in Bep production.

Methods: We combined global gene expression analyses with classic molecular and biochemical methods.

Results: We show that RpfR is a negative regulator of the Bep biosynthesis cluster and provide evidence that RpfR is not only able to degrade but also to synthesize c-di-GMP *in vitro* as well as *in vivo.* For full functionality of RpfR both the GGDEF and the EAL domain of the protein have to be intact. Our data suggest that the specificity of RpfR-mediated gene regulation is dependent on protein-protein interactions or subcellular localization rather than changes of the global cellular c-di-GMP pool.

O071 Staphylococcus aureus biofilms: understanding the role of cell wall anchored proteins

Joan Geoghegan[1]

[1]*Trinity College Dublin, Department of Microbiology, Ireland*

Staphylococcus aureus is an important human pathogen, often causing persistent, difficult to treat infections. *S. aureus* frequently adopts a biofilm phenotype in host tissues and on indwelling medical devices to resist immune and physical clearance mechanisms. Our understanding of the molecular interactions underlying biofilm development in staphylococci has advanced remarkably over the past decade. Cell wall anchored (CWA) proteins tether bacteria to host tissues or to indwelling medical devices that become conditioned with blood plasma proteins. The cell to cell interactions that occur during biofilm accumulation in *S. aureus* rely either on the production of a matrix of poly-N-acetyl-β-(1–6)-glucosamine or on cell surface-located proteins. The CWA proteins FnBPA, FnBPB, SasG and SdrC are each capable of forming interactions with identical partner proteins on a different cell linking the bacteria together in a biofilm. Alternatively CWA proteins promote biofilm formation through their interactions with host-derived molecules. For example, fibrinogen-binding proteins promote bacterial clumping in fibrin-rich fluids leading to the formation of biofilm-like aggregates. Here, recent insights into the protein-protein interactions underlying biofilm development in *S. aureus* will be described

O072 Biofilm-phage interaction dynamics and cell-cell interaction mechanics

Knut Drescher[1]

[1]*Max Planck Institute for Terrestrial Microbiology, Marburg, Germany*

Background: Biofilms are constantly exposed to phages in the natural environment, yet it is largely unknown which mechanisms govern their interactions.

Objectives: To understand principle mechanisms that determine the interactions of biofilms and phages, and how cells interact with each other in biofilms, new data acquisition and new data analysis methods are needed.

Methods: I will first present a new microscopy and imaging technique we recently developed to follow dynamical processes in biofilms at the single-cell level.

Results: I will then proceed to show how we applied this novel imaging technology in combination with genetics and molecular techniques to discover basic interaction mechanisms of biofilms and phages, how individual cells within biofilms interact with each other, and how biofilms disperse.

O081 Localization of the archaellum in haloarchaea

Sonja-Verena Albers[1]

[1]*Institute of Biology II, Microbiology, Molecular Biology of Archaea, Freiburg, Germany*

Background: The archaellum is a unique motility structure, which is evolutionary not related to bacterial flagella, but shows structural homologies to type IV pili. It achieves forward propulsion of the cells by an ATP driven rotary motor. We have analysed the subunit interaction of archaellum components from different archaeal species and performed detailed studies on archaellum assembly in the crenarchaeon *Sulfolobus acidocaldarius* and euryarchaeon *Haloferax volcanii*.

Objectives: We want to understand how and when the archaellum is assembled in haloarchaea during cell division.

Methods: Physiology, fluorescence microscopy, biochemistry

Results: *In H. volcanii* we could show that cells predominantly swim when they are rod-shaped in the early log phase. Using GFP-fusions we localized the archaellum at the poles and observed that the a new archaellum is formed prior to cell division at the new cell pole. Interestingly, euryarchaea have obtained the bacterial chemotaxis system by lateral gene transfer. In archaea it has to connect to the archaellum motor complex instead to the flagellar motor. Therefore, archaellum operons contain accessory proteins that enable CheY to interact with the archaellum motor complex. We studied the dynamics of different chemotaxis proteins and their interaction with motor components *in vivo* and *in vitro*.

O082 DNA Replication Without Origins

Thorsten Allers[1]

[1]*University of Nottingham, School of Life Sciences, Nottingham, United Kingdom*

Background: DNA replication is initiated at chromosomal sites called origins. Replication origins and the initiator proteins that bind them are assumed to be essential, but we have shown that in *Haloferax volcanii*, life without origins is not only possible but efficient.

Objectives: The replication enzymes found in archaea and eukaryotes differ fundamentally from those in bacteria; due to their shared evolutionary history, the former two are highly similar. For example, the archaeo-eukaryotic replicative helicase MCM is distinct to the bacterial replicative helicase DnaB. By contrast, the key enzyme for homologous recombination – known as RecA in bacteria, RadA in archaea and Rad51 in eukaryotes – is conserved in all domains.

Methods: If homologous recombination is an ancestral process that predates the split between bacteria, archaea and eukaryotes, and the evolution of their different machineries for DNA replication, could it have been used to initiate replication in the last common ancestor?

Results: We have shown that in *Haloferax volcanii*, deletion of all chromosomal origins leads to the initiation of all DNA replication by homologous recombination. Similar results have been obtained with deletion mutants lacking Orc1/Cdc6 replication initiator proteins, which are required for origin firing. Surprisingly, this leads to accelerated growth with no obvious defects, whereas deletion of origins (or initiator protein genes) in yeast or *Escherichia coli* leads to severe growth impairment. If homologous recombination alone can efficiently initiate the replication of an entire cellular genome, what purpose do replication origins serve and why they have evolved?

O083 Mechanisms of transcription in Archaea

Dina Grohmann[1]

[1]*University of Regensburg - Insititute of Microbiology, Archaea Centre, Regensburg, Germany*

Background: Archaeal transcription is carried out by a multisubunit RNA polymerase (RNAP) highly homologous to its eukaryotic counterpart RNAP II. Structures of RNAPs from all three domains of life were determined but they remained a static picture of a highly dynamic molecule.

Objectives: Understanding the conformational changes of the archaeal RNAP throughout the transcription cycle and the influence of transcription factors on RNAP activity and conformation. Gaining insights into transcription on the global scale in archaea.

Methods: Single-molecule fluorescence resonance energy transfer (FRET) measurements in conjunction with classical biochemical analysis and modern sequencing approaches.

Results:

(1) Dynamics of promoter DNA bending during initiation monitored in real-time: Archaeal TBP-promoter DNA interactions are highly dynamic and in some cases, archaeal transcription factor B is strictly required to bend the DNA leading to a regulatory scenario akin to sigma-dependent regulation.
(2) Conformational changes of the RNAP clamp during open complex formation: We studied the configuration of the main mobile element of the RNAP – the so-called clamp - throughout the transcription cycle using single-molecule FRET. This allowed us to access hitherto structurally uncharacterised states of the RNAP clamp and to discover the influence of transcription factors on the clamp conformation during the different phases of transcription.
(3) Direct detection of rRNA processing in Archaea: We have implemented and evaluated direct RNA sequencing via the Nanopore sequencing technology for the archaeal and bacterial model organisms. Among others, this allows us to follow the rRNA processing pathway and to delineate operon transcription patterns.

O084 Novel C-terminal lipid anchoring mechanism for prokaryotic surface proteins

Mechthild Pohlschroder[1], Mohd Farid Abdul Halim[1], Anthony DiLucido[1]

[1]*University of Pennsylvania, Biology, Philadelphia*

Microbial cell surface proteins play critical roles in many important biological processes, including mediation of intercellular communication, nutrient uptake, surface adhesion, motility, and maintaining and regulating, to a degree, cell shape. While many proteins are anchored via transmembrane (TM) domain insertion(s) into the membrane, some are anchored to the cell surface through covalent N-terminal links to a lipid moiety embedded in the membrane. Recently, a novel mechanism was discovered whereby a protein is anchored to the membrane through a lipid moiety covalently attached to a processed C-terminus. Both processing and lipid modification require an enzyme known as an archaeosortase (ArtA). Proteins recognized and processed by ArtA carry distinct C-terminal tripartite structures consisting of a conserved PGF motif, followed by a hydrophobic domain and then a stretch of positively charged residues. Molecular biological, biochemical, and microscopic analyses of the model archaeon *Haloferax volcanii* have confirmed the importance of the conserved PGF motif for ArtA-dependent processing, determined that ArtA processes a diverse set of proteins, including both Tat and Sec substrates, that play roles in processes such as motility and mating, and also revealed the active site of the ArtA peptidase. Moreover, two proteins homologous to enzymes required for archaetidylethanolamine biosynthesis are also critical for the processing and lipid-mediated anchoring of ArtA substrates. Since a broad array of archaea and bacteria encode either archaeosortases, or bacterial homologs known as exosortases - thus far studied only *in silico* – these findings have important implications for cell surface biogenesis in a wide variety of prokaryotes.

O085 Bacterial social strategies and their consequences

Ines Mandic-Mulec[1], Polonca Stefanic[1], Katarina Belcijan[1], Barbara Kraigher[1], Maja Bolješić[1], Iztok Dogsa[1], Tjaša Danevčič[1], Mihael Špacapan[1], Eva Kovacec[1]

[1]*University of Ljubljana, Biotechnical Faculty, Ljubljana, Slovenia*

Bacteria are perceived as social in that they communicate by chemical signals, organize into groups of cells such as biofilms or engage in group movements over surfaces such as swarming. They take part in fierce and unforgiving competitions for resources with rich arsenal of attack and defense strategies but also practice mutualistic (cooperative) behaviors through sharing of secreted molecules - public goods. Although it is becoming apparent that social phenotypes importantly contribute to ecological success of bacteria we are only beginning to grasp the power of bacterial sociality and intricate languages bacteria employ. The objective of the talk will be to provide an overview of bacterial social ecology with the focus on kin discrimination. One of the fundamental questions of social ecology is how bacteria decide whom to help and whom to attack? We have recently shown that soil isolates of *Bacillus subtilis* are capable of kin discriminations (KD), which is a differential treatment of related (kin) and less related (non-kin) individuals. By using *in vitro* synthetic communities and ecologically relevant plant root model we show that KD between *B. subtilis* genotypes encompasses competitive strategies between non-kin where non-kin are fiercely attacked while kin are embraced into a common group. KD shapes territoriality and spatial patterning of interacting genotypes, limits expansion of free riders and even promotes lateral gene transfer. The later has implications for ecological success of the evolved genotype and consequently may contribute to diversification of the species.

O086 Harnessing plant-Streptomyces interactions for the discovery of novel antimicrobials

Anne Van der Meij[1], Somayah Sameer Elsayed[1], Gilles P. van Wezel[1], Jos M. Raaijmakers[2], Joost Willemse[1]

[1]*Institute of Biology Leiden, Leiden, Netherlands*
[2]*Netherlands Institute of Ecology, Wageningen,*

Background: *Streptomyces* are filamentous bacteria that produce versatile natural products (NPs) relevant for agriculture, biotechnology and medicine, including the majority of the antibiotics we use in the clinic. Genome sequencing has unveiled that many antibiotic biosynthetic gene clusters are poorly expressed under routine laboratory conditions. This is a potential reservoir of untapped antibiotics that could be used to fight antimicrobial resistance. To identify them and elucidate their bioactivity, we need to uncover the triggers that elicit their production. Importantly, many *Streptomyces* live in symbiosis with eukaryotes, and in particular with plants. These interactions have likely played a key role in the evolution of the high chemical diversity of NPs[1].

Objectives: The STW Perspective program BacktoRoots aims to enhance plant growth and productivity by exploring and ultimately exploiting microbial communities[2]. The 'cry for help' hypothesis entails that plants suffering from biotic stress produce signals that activate the production of protective molecules by plant-associated microbes like *Streptomyces*. We aim to uncover these signals by studying plant-*Streptomyces* interactions and harness them for the screening of new antibiotics.

Methods: To study plant-*Streptomyces* interactions we made use of several imaging techniques, including electron microcsopy. Genes responding to plant hormones were found by RNA sequencing and molecular cloning.

Results: We were able to image the endophytic life-style of *Streptomyces*, as well as colonization and attachment of the spores to plant seeds[3]. In addition, we show that the plant-defense hormone jasmonic acid elicits the production of antibiotics, affects *Streptomyces* development and even may result in new phenotypes.

O087 Polymer breakdown through collective action of individuals in bacterial assemblages

Glen Dsouza[1;2], Vanessa Povolo[1;2], Martin Ackermann[1;2]

[1]*ETH Zürich, D-USYS, Zürich, Switzerland*
[2]*Swiss Federal Institute of Aquatic Science and Technology, D-UMIK, Dübendorf, Switzerland*

Background: In natural ecosystems, bacterial cells reside within spatial assemblages and often metabolize complex plant and animal derived nutrients like complex polysaccharides, in order to fuel essential cellular anabolic processes. As a consequence, growth and metabolism of cells brings about a transformation in the nutrient environment, which can strongly influence the behavior of other cells

Objectives: What growth behaviors manifest in the presence of complex polysaccharides? And how do these behaviors influence cellular metabolism?

Methods: We use microfluidics and time-lapse microscopy to study spatial and behavioral dynamics of individual cells associated with growth of the freshwater bacterium *Caulobacter crescentus* on xylan, a ubiquitous plant polysaccharide composed of recurring xylose units.

Results: In the presence of polymeric growth substrates, newly divided cells stay in close proximities of each other, thus effectively increasing size of a cell's neighborhood. In contrast, cells grown in presence of the monomer, xylose, largely lead solitary lives. We find that such collective behavior in the presence of xylan is beneficial for individual cells for two reasons: first, to avoid a diffusional loss and thus to potentially increase the availability of both, the exoenyzme and end product: i.e. xylanase and xylose. Second, collective growth results in cells which are born in a microcolony to progressively increase their growth rates, a possible benefit of being in the proximity of other polysaccharide degrading cells. Our results elucidate the advantages of growth in spatially structured environments and potentially shed light on a likely ubiquitous and adaptive metabolic behavior in microbial assemblages.

O088 Bacillus lifestyle in the tough rhizosphere world: some talent for tailoring its secondary metabolome

Anthony Argüelles Arias[1], Gregory Hoff[1], Sofija Andric[1], Dong Xue[1], Thibault Meyer[1], Victoria-Francisca Osorio Torrens[2], Nicolas Smargiasso[2], Edwin De Pauw[2], Marc Ongena[1]

[1]University of Liege, Microbial Processes and Interactions Lab, TERRA Research Center, Gembloux Agro-Bio Tech Faculty, Gembloux, Belgium
[2]University of Liege, Mass Spectrometry Laboratory (LSM-GIGA-R), Chemistry Department, Liege, Belgium

Background: Some isolates belonging to the plant-associated *Bacillus velezensis* species are among the most promising bacteria to be used as biocontrol agents to protect plants against diseases in sustainable agriculture. These bacteria are particularly rich in genes devoted to the synthesis of secondary metabolites retaining key natural functions for establishment and persistence in the highly competitive rhizosphere ecological niche. Most compounds in the secretome of *B. velezensis* are best characterized as antimicrobials to protect against competitors but some are seemingly more multifunctional and also retain signaling functions.

Objectives: Our global aim is to better appreciate how far this arsenal of bioactive molecules is actually expressed *in planta* and to what extent this can be modulated by rhizosphere-inherent factors such as abiotic stress and interspecies/interkingdom cross talks.

Methods: The production of secondary metabolites was determined by combining targeted transcriptomics (qPCR, GFP-tagged derivatives) and metabolomics (UPLC-HR-MSMS, Imaging MS).

Results: The production of different categories of compounds is specifically stimulated by *Bacillus* according to the interacting partner or in response to cold stress. For instance, interaction with the host plant drives the bacterium to very efficiently produce the surfactin lipopeptide, which clearly contributes to its rhizosphere fitness. Upon perception of *Pseudomonas*, *B. velezensis* specifically stimulates the formation of some polyketides and lantibiotics with antibacterial activity and in confrontation with fungi or oomycetes, *Bacillus* boosts fengycin lipopeptides retaining strong antifungal activity. These results illustrate the potential of rhizosphere-dwelling bacilli to re-direct their secondary metabolome in an appropriate way to ensure ecological persistence.

O089 Message in a bubble - Communication via extracellular vesicles shapes algal bloom dynamics

Daniella Schatz[1], Guy Schleyer[1], Marius R. Saltvedt[2], Shilo Rosenwasser[3], Ester Feldmesser[4], Janice Lawrence[5], Ruth-Anne Sandaa[2], Assaf Vardi[1]

[1]*Weizmann Institute of Science, Department of Plant and Environmental Sciences, Rehovot, Israel*
[2]*University of Bergen, Department of Biological Sciences, Bergen, Norway*
[3]*The Hebrew University of Jerusalem, The Robert H. Smith Institute of Plant Sciences and Genetics in Agriculture, Rehovot, Israel*
[4]*Weizmann Institute of Science, Department of Life Sciences Core Facilities, Rehovot, Israel*
[5]*University of New Brunswick, Department of Biology, New Brunswick, Canada*

Background: Communication between microorganisms in the marine environment has immense ecological impact by mediating trophic-level interactions and thus determining community structure. Extracellular vesicles (EVs) are produced by cell from all kingdoms of life and can mediate pathogenicity or act as vectors for intracellular communication. However, little is known about the involvement of EVs in microbial interactions in the marine environment.

Objectives: We investigated the signaling role of EVs produced during the interaction between the bloom-forming alga *Emiliania huxleyi* and its specific virus, EhV, which leads to demise of these large-scale oceanic blooms.

Methods: We isolated EVs using filtration and density gradients, characterized them by lipidomics transcriptomics and proteomics, and applied them to natural populations, infected cultures or virions to test for their biological activity.

Results: Production of EVs is elevated during viral infection or when cells are exposed to infochemicals derived from infected cells. These vesicles have a unique composition that differs from that of viruses and their infected host cells. Their cargo is composed of specific small RNAs that are predicted to target sphingolipid metabolism and cell-cycle pathways. In both lab and field experiments we demonstrated that treatment with EVs leads to a faster viral infection dynamic. EVs can prolong the half-life of both isolated and natural EhV virions. We propose that extracellular vesicles are exploited by viruses to sustain efficient infectivity and propagation across *E. huxleyi* blooms. Therefore, EVs may influence the fate of the blooms and the composition and flow of nutrients through marine microbial food webs.

O090 The colonization capacity of exogenous Lactobacillus plantarum depends on human gut microbiota composition in vitro

Isenring Julia[1], Stevens Marc J.A.[2], Geirnaert Annelies [1], Hall Alex[3], Lacroix Christophe[1], Jans Christoph[1]

[1]ETH Zurich, D-HEST, IFNH, Food Biotechnology, Zürich, Switzerland
[2]University of Zurich, Institute for Food Safety and Hygiene, Zürich, Switzerland
[3]ETH Zurich, Institute of Integrative Biology, Zürich, Switzerland

Background: Humans continuously ingest microorganisms at high numbers via food or environment. Their dynamic behavior within the complex gut microbiota remains however unknown due to inaccessibility of the gut microbiota. Continuous *in vitro* gut fermentation models offer the opportunity to monitor colonization of such exogenous strains and to investigate individual strain interactions within complex ecosystems.

Objectives: Here we used a state-of-the-art *in vitro* model for the human gut microbiota to study the colonization capacity of the exogenous model strain *Lb. plantarum* NZ3400Cm.

Methods: Two different human fecal microbiota were continuously cultivated over three months under human colon conditions using the PolyFermS model. It comprised of an inoculum reactor containing immobilized fecal microbiota, inoculating five second-stage reactors, where *Lb. plantarum* was added in a single dose. Microbiota composition, metabolite production, and *Lb. plantarum* (selective plating) were monitored daily.

Results: Addition of *Lb. plantarum* (10E9 CFU/ml) led to steady but different colonization (tested up to 2 months) in donor 1 (10E3 CFU/ml) and donor 2 (10E5 CFU/ml) microbiota, four days after supplementation. The colonization capacity was not affected by the addition level (10E9 and 10E6 CFU/ml). A second supplementation did not increase strain abundance. Furthermore, *Lb. plantarum* colonization did not induce detectable changes in end metabolite production of carbohydrate fermentation. Our data showed colonization capacity of *Lb. plantarum* in an *in vitro* model of colonic microbiota and suggests that colonization is likely determined by a donor-microbiota-specific niche. This study also demonstrated the potential of the PolyFermS intestinal model to elucidate strain-microbiota interactions.

O091 Identification of the parameters driving Contact-Dependent Inhibition induced spatial organisation within Bacterial populations

Jamie Wood[1]

[1]*York, Mathematics/Biology, York, United Kingdom*

Background: Identifying how microbes are able to manipulate, survive and thrive in complex multispecies communities has expanded our understanding of how microbial ecosystems impact human health and the environment. The ability of bacteria to negatively affect neighbours, through explicit toxin delivery systems, provides them with an opportunity to manipulate microbial communities to their benefit. Contact-dependent inhibition (CDI) systems (members of Type Vb class secretion systems) are a distinct subset of competition systems whose contribution to shaping the spatial structure of bacterial communities is yet to be fully understood.

Objectives: In this talk I will compare the impact of different CDI systems, at both the single cell and population level, to determine the key drivers of CDI-induced spatial organisation within bacterial populations.

Methods: Through an iterative approach using both an E. coli experimental system and computational modelling, we show that CDI systems have subtle and system-specific effects at the single cell level, generating significant differences in their contribution to the spatial distribution of bacterial populations on a solid surface.

Results: In addition to the general effect of seeding density, we show that the cost of CDI expression, and importantly the CDI specific parameter of rate of inhibition, significantly impacts the spatial pattern of the inhibitor and target strain. In contrast, the magnitude of the toxin-induced growth retardation of target cells only weakly impacts organisation of the population. Our work reveals how distinct CDI systems can differentially modify, and potentially control, the spatial arrangement within bacterial populations.

O092 Molecular Effectors of TB Pathogenesis

Patrick Brennan[1]

[1]*Colorado State University, Microbiology, Immunology and Pathology, Fort Collins, United States*

Background: As recently as 2017, *Mycobacterium tuberculosis* caused 1.6 million deaths, and initiated approximately 10.0 million new cases of tuberculosis worldwide (WHO, 2016). Understanding the host-pathogen interactions of *Mycobacterium tuberculosis* on a molecular level is vital to decreasing the global burden of tuberculosis disease

Objectives: Understanding the host-pathogen interactions of *Mycobacterium tuberculosis* on a molecular level is vital to decreasing the global burden of tuberculosis disease

Methods: In a collection of MiniReviews, commissioned and edited by Guest Editors Kathleen McDonough and Patrick Brennan and published in issues of *Pathogens and Disease (PAD)* throughout 2018, current knowledge of the field, and potential future developments, were explored from the perspectives of the roles of: cholesterol; extracellular vesicles; macrophage manipulations; toxins/anti-toxins; cyclic nucleotides; the stringent response; among several other topics

Results: To be discussed in this present presentation, how recent impressive progress in definition of the structure, biogenesis, genetics and gene manipulations of cell wall components of *M. tuberculosis* allows insights into the role in the pathogenesis of *M. tuberculosis* of such crucial cell wall entities as: phthicerol dimycocerosate/"attenuation factor"; trehalose dimycolate/"cord factor"; sulfolipids; lipoarabinomannan and its precursors; lipooligosaccharides; even the recently discovered minor modifications of the cell wall core, peptidoglycan-arabinogalactan. The outcome of this presentation in conjunction with the recent Thematic Issue in *PAD* will result in a comprehensive perspective of the variety and complexity of the molecular effectors resulting in the pathology of a global disease still of profound significance in terms of morbidity and mortality

O093 Development of a Human challenge model for Tuberculosis

Brian Robertson[1], Nitya Krishnan[1], Natalie Charitakis[1], Miles Priestman[1], Iria Castro[1], Thomas Baer[2]

[1]*Imperial College London, Medicine, London, United Kingdom*
[2]*Stanford University*

Background: The development of new TB vaccines to replace or boost BCG has been hampered by the lack of tractable animal models that reproduce key elements of human infection, and the paucity of biomarkers that report on protective immunity. The development of a human challenge model for tuberculosis could aid in TB vaccine development by measuring vaccine efficacy in humans in small scale trials using anti-mycobacteria immune responses as the read out.

Objectives: To construct mycobacterial reporter strains suitable for intradermal detection, and demonstrate loss of signal in response to vaccination or drug treatment using non-invasive imaging.

Methods: Fluorescent BCG expressing red and yellow reporters were constucted and tested in a mouse vaccination model, using a novel LED-based imaging system to capture fluorescent images non-invasively over time.

Results: We have developed a skin-based challenge model using fluorescent BCG as the reporter strain, and a novel imaging system which captures fluorescent images non-invasively over time. Data will be presented showing the rapid loss of fluorescent signal over time both after drug treatment, and after BCG vaccination.

This provides the basis for future work using an attenuated *M. tuberculosis* strain and alternative vaccines, and ultimately a skin-based human challenge model.

O094 Evolution of virulence in the Mycobacterium tuberculosis complex

Roland Brosch[1]

[1]*Institut Pasteur, Genomes and genetics, Paris, France*

Background: Mycobacterium tuberculosis, the causative agent of human tuberculosis is one of the most widely spread human pathogens. It is estimated to have infected a quarter of the global human population by developing specialized ways to circumvent innate and adaptive host immune responses. This highly adapted, major human pathogen is thought to have evolved from a pool of ancestral environmental mycobacteria, whose extant representatives are known under the name of Mycobacterium canettii.

Objectives: The objectives of our work are to better understand the virulence determinants of human tubercle bacilli by analysing the genome sequences of different Mycobacterium tuberculosis and Mycobacterium canettii strains in relation with results from selected virulence evaluation models.

Methods: Our methods consist of genome sequence analyses, phylogenetic analyses, virulence determination in selected infection models and genetic manipulation for producing and testing recombinant mycobacteria that can be then tested for their different phenotypes in selected models.

Results: Gain and loss of genetic regions has been key to the outstanding evolutionary success of the tuberculosis agent. Here, selected, recently elucidated examples will be presented and discussed.

O095 How the first-discovered mycobacterial virulence protein Erp functions

Lalita Ramakrishnan[1]

[1]*Cambridge University, United Kingdom*

In 1998, the mycobacterial protein Erp, a cell surface component, was the first *Mycobacterium* virulence protein to be identified by targeted deletion. Erp is required for mycobacterial growth in macrophages and virulence. However, its role in virulence remains poorly understood. I will present new functional, biochemical and structural data that suggest its virulence mechanism and discuss their implications.

O096 African swine fever virus inhibition of type I interferon and apoptosis

Linda Dixon[1], Ana Reis[1], Samuel Connell[1], Anusyah Rathakrishnan[1], Lynnette Goatley[1], Lauren Gates-Tanzer[2], Claire Barber[1], Stephen Goodbourn[3], Joanna Shisler[2]

[1]The Pirbright Institute, ASFV, Woking, United Kingdom
[2]University of Illinois at Urbana-Champaign, Champaign, United States
[3]St George's, University of London, United Kingdom

Background: African swine fever virus (ASFV) causes an economically important disease of pigs and wild boar that can result in case fatality approaching 100%. ASFV is the only member of the *Asfarviridae* family and replicates in the cell cytoplasm The virus genome contains 150 to 167 proteins open reading frames including many that are not essentail for replication in cell culture. Many of these code for inhibitors of the host innate immune response including inhibitors of the type I interferon (IFN) response and apoptosis. Deletion of a number of these genes has been shown to reduce virus virulence in pigs.

Objectives: The objectives were to: 1 Identify additional ASFV encoded inhibitors of type I IFN and apoptosis. 2. Determine the mechanisms by which these proteins function and their roles during virus infection of macrophages and pigs.

Methods: ASFV encoded proteins that inhibit induction of reporter genes dependent on promoter elements from the IFN beta promoter (IRF3 and NF-κB) were screened for. In a separate screen inhibitors of the stress induced pro-apoptotic CHOP pathways were identified. Attempts were made to delete the genes coding for the proteins from the genome of cell culture adpated or highly virulent ASFV isolates.

Results: ASFV proteins from multigene families 360 and 505 and others were shown to inhibit induction of IRF3 and NF-kB dependednt luciferase reporters and their targets in the relevant signalling pathways identified. Three inhibitors of CHOP were identified. Gene deletions from virulent ASFV resulted in virus attenuation in pigs.

O097 cbp80/cbp20-dependent translation initiation factor (ctif) inhibits hiv-1 gag synthesis by targeting the activity of the viral protein rev

Francisco García-de-Gracia[1], daniela toro-ascuy[1], camila pereira-montecinos[1], aracelly gaete[1], barbara royas-araya[1], sebastian riquelme[1], fernando valiente-echeverria[1], ricardo soto-rifo[1]

[1]Universidad de Chile, Molecular and Cellular Virology Laboratory, Virology Program, Institute of Biomedical Sciences, Faculty of Medicine, Santiago, Chile

Background: The Human Immunodeficiency Virus type-1 (HIV-1) Gag polyprotein is synthetized from the unspliced mRNA in a process involving a non-canonical nuclear export pathway as well as cap-dependent and cap-independent mechanisms of translation initiation. The viral protein Rev is critical in ensuring proper Gag synthesis during viral replication by acting as the master post-transcriptional regulator of the unspliced mRNA. Previous data have suggested that translation of the unspliced mRNA occurs by a cap-dependent, CBC-driven mechanism, which is active during a virally-induced inhibition of eIF4E activity. In addition, it was show that Rev interacts with the CBC subunit CBP80, suggesting that Rev is involved in CBC-mediated translation. However, the role of other components of the CBC-dependent translation initiation complex is unknown.

Objectives: The aim of this study is determinate the role of CBP80/20-dependent translation initiation factor (CTIF) protein in the synthesis of Gag.

Methods: We used infection assay, overexpression and know down of CTIF, proximity ligation assay and immunofluorecense.

Results: We show that CTIF acts as a strong inhibitor of Gag synthesis by interfering with the activity of the viral protein Rev. We also report that the N-terminal, CBP80-binding domain of CTIF, contains all the determinants required for this inhibitory activity. As such, both full-length CTIF and the CBP80-binding domain interacts with Rev inducing the retention of the viral protein in the cytoplasm. We observed the same inhibitory effect of CTIF in the synthesis of Gag of HIV-2.

O098 Hijacking the hijackers: Escherichia coli phage-inducible chromosomal islands redirect lambda phage packaging for their own benefit

Alfred Fillol-Salom[1], Julio Bacarizo Roa[1], Mohammed Alqasmi[1], Alberto Marina[2], Jose R Penades Casanova[1]

[1]*Institute of Infection, Immunity and Inflammation, Bacteriology, Glasgow, United Kingdom*
[2]*IBV, Unidad cristalografica, Valencia, Spain*

Background: Phage-inducible chromosomal islands (PICIs) are clinically relevant mobile genetic elements widespread among Gram-positive and Gram-negative bacteria. Understanding the different PICI packaging mechanisms is essential to comprehend their intra- and inter-generic transfer, which contribute to bacterial evolution, host adaptation and pathogenesis.

Objectives: Hijacking and blocking phage functions is vital for the PICI molecular pirates. They usually encode a battery of genetic resources to interfere with the phage. The objective of this study was to characterise the mechanism employed by EcCICFT073 to hijack the phage TerS, redirecting its affinity to package the PICI dsDNA.

Methods: We identified by *in vivo* (phage evolution, genetic scarless mutations, competition assays) and *in vitro* (Two-hybrid assays, X-ray crystallography) experiments the molecular mechanism by which EcCICFT073 recruits the phage packaging machinery.

Results: This study identified a novel and elegant one-shot strategy used by some *cos* lambda *E. coli* PICIs like EcCICFT073. Unlike other *cos* PICIs that carry the same phage *cos* sequence, EcCICFT073 carries the lambda phage *cos*Q and *cos*N, but a different *cos*B region (region involved in packaging initiation). Hence, these PICIs have developed a sophisticated strategy by encoding a protein, renamed as Rpp (Redirecting phage packaging) which forms a heterodimer with the phage TerS to perform dual roles: i) forming a new functional hetero DNA-binding (DBD) region that will be used to recognise the PICI *cos*B site; and ii) this new DBD would be unable to recognise the phage *cos* site, blocking phage packaging. This novel strategy highlights PICIs as sophisticated parasites in nature.

O099 Epitranscriptomic regulation of HIV-1 gene expression

Bryan Cullen[1]

[1]*Duke University Medical Center, Molecular Genetics and Microbiology, Durham, NC, United States*

Background: While it has long been known that a range of virally encoded RNAs, including viral mRNAs, bear multiple covalently modified nucleotides, the functional consequences of these epitranscriptomic modifications have remained unclear.

Objectives: Quantify and map the epitranscriptomic modifications found on HIV-1 RNA and determine their effect on viral replication and gene expression

Methods: We used UHPLC MS/MS to quantify modified nucleotides on highly purified viral RNA and a range of molecular biological techniques to determine their phenotypic effect.

Results: Analysis of purified HIV-1 genomic RNA demonstrates the presence ~14 A residues methylated at the N6 position (m^6A), ~11 C residues methylated at the C5 position (m^5C) and >60 nucleotides modified by 2'O methylation of the ribose moiety. Using overexpression and/or genetic ablation strategies, as well as by mapping and mutational inactivation of specific viral mRNA m^6A addition sites, we previously demonstrated that m^6A residues boost HIV-1 replication. We have now extended this work by mapping the m^5C residues found on HIV-1 genomic RNA and mRNA, and by identifying the primary m^5C "writer" as cellular NSUN2 and the m^5C "reader" as MBD2. Importantly, loss of NSUN2 function not only prevents m^5C addition to HIV-1 transcripts but also inhibits HIV-1 replication by reducing HIV-1 mRNA translation and by deregulating alternative splicing of HIV-1 mRNAs. These data therefore identify m^5C as a second epitranscriptomic modification that promotes HIV-1 replication.

O100 Unveiling the molecular mechanism of phage arbitrium communication system

Alberto Marina[1;1], Francisca Gallego del Sol[1], Jose Penades[2]

[1]*Instituto de Biomedicina de Valencia (CSIC), Genomic and Proteomic, Valencia, Spain*
[2]*Institute of Infection, Immunity and Inflammation, University of Glasgow, Glasgow, United Kingdom*

Background: Bacteriophages can control their life cycles via density-dependent lytic-lysogenic decisions. This decision severely impacts both viral and bacterial ecology and evolution. It has been recently described that *Bacillus* phages use a communication system, termed “arbitrium”, to coordinate lysis-lysogeny decisions. Arbitrium system communicates by producing a hexapeptide (AimP) during their lytic cycle that is secreted to the medium. Once internalized, the peptide reduces the expression of the negative regulator of lysogeny AimX by binding to the transcription factor AimR, promoting lysogeny. Thus, the relative population of active phages correlates with the concentration of AimP, determining whether the phages enter the lytic or the lysogenic cycle.

Objectives: To understand the molecular basis of arbitrium communication system,

Methods: We have elucidated the crystal structures of AimR from the *Bacillus subtilis* SPbeta phage in its apo form, bound to its DNA operator and in complex with its cognate AimP regulator peptide.

Results: The structures showed that AimR presents intrinsic plasticity, sharing structural features with the RRNPP quorum sensing family. Remarkably, AimR binds to an unusual operator with a long 25 bp spacer that interacts nonspecifically with the receptor TPR domain, while the HTH domain canonically recognizes two inverted repeats. AimP stabilizes a compact conformation of AimR that approaches the DNA-recognition helices, preventing AimR binding to its cognate promoter region. Intriguingly the AimR DNA-recognition helices remain in a compatible DNA-binding conformation in the AimR-AimP structure, opening the possibility of additional regulatory activities of AimR in its peptide-bound state.

O101 A new platform to visualise the effects of host proteins on HIV-1 capsid uncoating at the single-particle level

Chantal Marquez[1], Stuart Turville[2], Till Boecking[3]

[1]Molecular and Cellular Virology Laboratory, Institute of Biomedical Sciences, Faculty of Medicine, Universidad de Chile, Santiago, Chile
[2]Kirby Institute, UNSW Sydney, Sydney, Australia
[3]EMBL Australia Node in Single Molecule Science and ARC Centre of Excellence in Advanced Molecular Imaging, School of Medical Science, UNSW Sydney, Sydney, Australia

Background: A key step in the HIV-1 infection cycle is the disassembly of the capsid shell that protects the viral genome, a process referred to as "uncoating". Recent studies have shown that host cell factors regulate uncoating, but the complete mechanism remain unclear. The fragility of the capsid has so far hindered the development of methods to detect single steps in the process.

Objectives: Here, we have developed a novel fluorescence microscopy method to follow the real-time uncoating kinetics of individual HIV capsids to study the binding of proteins and assess their effect on uncoating.

Methods: GFP-loaded viral particles are permeabilized with a pore forming protein and we (1) pinpoint the first defect of the capsid by detecting the GFP release, (2) follow the disassembly kinetics of the remainder of the lattice by "painting" it with a fluorescent marker, and (3) extract binding kinetic parameters of CA-binding proteins with the core.

Results: Our results show that spontaneous HIV-1 uncoating is initiated very soon after permeabilization of the viral envelope. Opening of the lattice is the rate-limiting step of uncoating, which is followed by rapid, catastrophic collapse. Addition of the capsid-binding inhibitor PF74 accelerates capsid opening but stabilizes the remaining lattice, while the binding of IP6 strongly delays core opening but does not prevent lattice disassembly thereafter. Our observations suggest that HIV-1 capsid uncoating can be dissected in two steps, capsid opening and lattice disassembly and that they can be controlled independently by different capsid-binding regulators.

O102 Dengue virus NS5 polymerase counteracts PML-mediated intrinsic immunity

Federico Giovannoni[1], Maria Fatima Ladelfa[1], Martin Monte[1], David Jans[2], Peter Hemmerich[3], Cybele Garcia[1]

[1]School of Sciences, University of Buenos Aires, Argentina
[2]Monash University, Clayton, Australia
[3] Leibniz Institute on Aging – Fritz Lipmann Institute, Jena, Germany

Background: Dengue fever, caused by dengue virus (DENV) infection, is an important public health problem threatening 40% of the world's population. Currently there is no antiviral therapy against DENV; its development requires a better understanding of virus-host interactions and antiviral immunity. Besides innate and adaptive immunity, intrinsic immunity in individual cells plays a key role in the control of viral infections. Intrinsic immunity depends on cellular proteins that block viral replication at any stage of the virus life cycle. Promyelocytic leukemia (PML) protein contributes to intrinsic immunity against many viruses and is the key organizer of nuclear bodies (PML-NBs), which are discrete structures within the nucleus of mammalian cells. There is increasing evidence involving PML as a DNA virus antiviral factor, but less information is available regarding the role of PML against RNA viruses, including DENV.

Objectives: Characterize the role of PML as a mediator of intrinsic immunity against DENV.

Methods: A549 cells and the four different serotypes of DENV were used throughout the study.

Results: By performing knock-down and overexpression studies, we demonstrated that PML displays antiviral activity against DENV. We showed that, in infected cells, PML-NBs are disassembled after DENV infection and we proved that the DENV polymerase (non-structural protein 5, NS5) interacts with PML to disrupt PML-NBs and counteract PML-mediated intrinsic immunity. These data are expected to contribute to the understanding of the biological principles underlying DENV strategy to evade the host antiviral response.

O103 Isolation and characterization of temperature sensitive mutants of mycobacteriophage d29

Shrestha Ghosh[1]

[1]*Bose Institute, Microbiology, KOLKATA, India*

Background: Mycobacteriophage D29 is a lytic phage which infects various species belonging to the genus *Mycobacterium* which includes the causative agent of tuberculosis. D29 phage exits the infected host cell by a lytic process. Temperature sensitive (ts) mutants which do not produce plaques at the non- permissive temperature (42°C) are cornerstones of molecular biology, particularly in the case of Mycobacteriophage meta-genome, which is at present, a veritable black box. The genome of this phage comprises as many as 80 open reading frames (ORFs). However apart from a few proteins encoded by these ORF, the function of most are unknown. Though for some proteins *in silico* leads are available, only a small number has been bio-chemically characterized.

Objectives: Our objective is to undertake a mutational approach towards understanding how mycobacteriophage D29 proteins function.

Methods: The methodology adopted is a forward genetics approach in which the phage was mutated randomly using hydroxylamine resulting in the inactivation of open reading frames without bias followed by transcription analysis of the mutants.

Results: Several thermosensitive mutants of the mycobacteriophage D29 were obtained. The mutants were characterized using temperature shift assays, growth curves, and transcriptional analysis of infected host bacteria at non-permissive temperature. We were able to characterize two mutants, namely ts10 and ts12.Temperatureshift assays and transcriptomic analysis revealed that ts10 is an early mutant due to low transcript abundance of early genes (gp89) whereas ts12 is a late mutant due to low abundance of a late gene (gp12) which is a lysin.

O104 Shedding-light on the mechanical properties and structural changes of E. coli following T4 phage infection

Edo Bar-Zeev[1], Shiju Abraham[2], Yair Kaufman[2]

[1]*Zuckerberg Institute for Water Research, midreshet ben-gurion, Israel*
[2]*Zuckerberg Institute for Water Research, Israel*

Background: Viruses, are ubiquitous nano-parasites that infect a wide spectrum of hosts, ranging from humans to archaea, with bacterial viruses (phages) being the focus of this study. Significant efforts were previously made to study the mechanisms that take place during *E. coli* phage infection. Yet, the changes in the mechanical properties and cell topography of *E. coli* during phage infection were not explored.

Objectives: The overarching goal was to study in-real time the structural and mechanical changes of *E. coli* biofilms during phage infection.

Methods: Initially, *E. coli* cells were immobilized and grown on a positively charged lipid bilayer that was prepared on a glass coupon. Nano-scale images of the cell wall structure were captured in real-time using atomic force microscopy (AFM). Concomitantly, lyses of the cells were visualized by live/dead tagging while viscoelasticity and deformation of the cells were measured *in-situ* with the bio-force spectroscopy.

Results: Our results indicate that after one hour of T4 infection the outer-membrane of *E. coli* cells was highly perforated, exhibiting cavities of up to 350 nm in diameter (Fig. 1). Within that first hour of T4 infection, *E. coli* cells were structurally intact, yet significantly (1 fold) harder. During the second hour the *E. coli* cells were perforated and exhibit softer behavior 2-800 KPa, following complete cell lysis. We surmise that these results shed new light on *E. coli* infection which could provide additional information toward *E. coli* biofilm control.

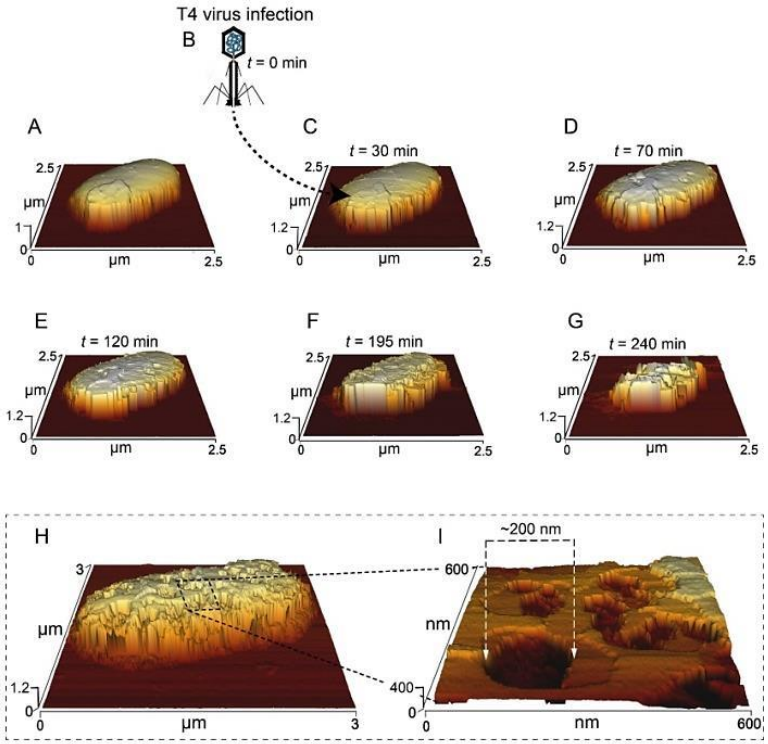


Figure. 1. 3D topography of *E. coli* cells before and after T4 infection.

O105 Beyond CRISPR: The immune system of bacteria

Rotem Sorek[1]

[1]*Weizmann Institute of Science, Department of Molecular Genetics, Rehovot, Israel*

Background: The arms race between bacteria and phages led to the development of sophisticated anti-phage defense systems, including CRISPR-Cas and restriction-modification systems. However, evidence suggest that many yet unknown defense systems reside in microbial genomes.

Objectives: Our aim is to systematically characterize the bacterial defense arsenal against phages.

Methods: For this, we examine gene clusters found within "defense islands" in >45,000 bacterial and archaeal genomes. Candidate systems are tested within model bacteria for their ability to provide resistance against a wide array of phages.

Results: We discovered a large number of anti-phage defense systems, including systems that adopted components of the bacterial flagella and condensin complexes, as well as systems suggesting an ancient ancestry of innate immunity components shared between animals, plants, and bacteria. The talk will describe progress in mechanistic understanding of these recently discovered systems.

O106 Genome hypermobility by lateral transduction

Jose Penades[1]

[1]*University of Glasgow, Glasgow, United Kingdom*

Background: For the first time in 60 years, and using the pathogen *Staphylococcus aureus* as a model, we have discovered a new mechanism of genetic transduction. This new mechanism, which we have named lateral transduction, is at least 1,000 times more efficient than any currently known mechanism of transduction, with the ability to transfer large sections of bacterial chromosomes (several hundred kilobases) between bacteria at extremely high frequencies. In fact, lateral transduction elevates the concept of mobile genetic elements well beyond that of defined DNA elements, by transforming sections of the genome into hypermobile platforms that are capable of transferring any genetic element within their boundaries at incredibly high frequencies. The discovery of lateral transduction could help to explain the rapid evolution of bacteria that occurs, for example, in the development of multi-drug resistant strains. Since phages are by far the most abundant biological entities on the planet, the importance of genetic transduction as one of the principal drivers of microbial evolution has never been more apparent than with the discovery of lateral transduction.

Results: Our recent discovery challenges the perception about the impact that phages may have on bacterial evolution. It is therefore imperative to address critically an important question surrounding this process: is lateral transduction exclusive to *S. aureus* phages or is it widespread in nature? We will answer here this question, providing new mechanisms involving bacteriophages in bacterial evolution, including antimicrobial resistance.

O107 Why so many types of mobile genetic elements?

Eduardo Rocha[1;2]

[1]*Institut Pasteur, Genomics and Genetics, Paris, France*
[2]*CNRS UMR 3525, Paris, France*

Background: Evolutionary processes are typically described as the result of mutation, descent and selection. Many microbes lack sexual reproduction but have the ability to acquire genetic information from very distantly related organisms. Horizontal gene transfer allows the instantaneous acquisition of new complex adaptive traits and their transmission to subsequent generations. This speeds up evolutionary processes as exemplified by the acquisition of virulence traits in emerging infectious agents and by antibiotic resistance in many human bacterial pathogens.

Objectives: Understand why there are so many different types of MGE

Methods: Comparative genomics, modeling, evolutionary methods.

Results: I'll describe how differences between mobile genetic elements in terms of mechanism of transmission between cells and mechanisms of stabilization within cells can shape their lifestyle and affect the traits they spread in bacterial communities.

O108 Comparative transcriptomics identifies a single SNP mutation that controls virulence of African Salmonella

Jay Hinton[1]

[1]*Institute of Integrative Biology, Liverpool, United Kingdom*

Background: With 3.4 million infections each year, invasive non-Typhoidal Salmonella (iNTS) is a major cause of illness worldwide. In Sub-Saharan Africa, which carries the heaviest iNTS burden, bloodstream infections with iNTS Salmonella enterica serovar Typhimurium are causing ~388,000 deaths annually. Co-infection with HIV or malaria in adults, and a young age (<5 years) are known risk factors.

Objectives: Whole-genome sequencing of iNTS strains revealed a new sequence type (ST313) that showed high similarity to the well-studied non-invasive S. Typhimurium ST19 responsible for gastroenteritis. The core genomes of two representative strains of ST313 (D23580) and ST19 (4/74) differ by 856 SNPs. We hypothesized that phenotypic differences between strains D23580 and 4/74 would reveal the properties of ST313 responsible for invasive human disease.

Methods: The primary transcriptomes of D23580 and 4/74 were defined by a combined RNA-seq/dRNA-seq approach (Kröger et al., 2013), and we used the transcriptomic data to compare expression of bacterial promoters. We used functional transcriptomics and proteomics to discover phenotypic differences that distinguish African from global Salmonella pathovariants (Canals et al., 2019)

Results:
Our analysis led us to identify a single core genome SNP responsible for the up-regulation of a promoter in strain D23580 that controlled the expression of the S. Typhimurium PgtE virulence factor (Hammarlöf et al., 2018). We propose that the ability of S. Typhimurium ST313 to express high levels of PgtE contributes to bacterial survival during systemic infection, and has contributed to the pan-African epidemic of bloodstream infection.

References

Canals et al. (2019) Adding function to the genome of African Salmonella Typhimurium ST313 strain D23580. PLoS Biology 17(1): e3000059.

Kröger et al. (2013) An Infection-relevant transcriptomic compendium for Salmonella enterica serovar Typhimurium. Cell Host & Microbe 14: 683–695.

Hammarlöf et al. (2018) Role of a single noncoding nucleotide in the evolution of an epidemic African clade of Salmonella. Proc. Nat, Acad. Sci. USA 115: E2614 - E2623.

O109 The role of genetics and evolution in Staphylococcus aureus infection

Daniel Wilson[1]

[1]*University of Oxford, Big Data Institute, Oxford, United Kingdom*

Background: Like other major bacterial pathogens, *S. aureus* asymptomatically colonizes the human body far more frequently than it causes severe infections.

Objectives: This raises the question of what are the evolutionary and genetic differences between infection and asymptomatic colonization?

Methods: To address this question we have sequenced and analysed *S. aureus* genomes to chart evolution within hundreds of healthy and infected patients, and to discover genetic differences associated with bacteria infecting and colonizing thousands of unrelated cases and controls.

Results: These studies reveal surprising differences in the genetic basis of different types of infection, and reveal signatures of bacterial adaptation within the human body to selection pressures that are as yet incompletely understood.

O110 Role of the microbiome in the defense against antibiotic resistant pathogens

Carles Ubeda[1]

[1]*FISABIO, Genómica y Salud, Valencia, Spain*

Background: Antibiotic resistant pathogens (ARPs), such as vancomycin-resistant Enterococcus (VRE) or multidrug-resistant Enterobacteriaceae (MRE) are an increasing problem in hospitalized patients. Infections with ARPs frequently begin by colonization of the intestinal tract. In normal conditions our microbiome suppresses intestinal colonization by ARPs. However, antibiotic treatment alters the microbiome and enables ARPs to densely colonize the gut, which promotes ARP dissemination to the bloodstream and transmission to other patients.

Objectives: The identification of commensal bacteria that are key for suppressing ARP intestinal colonization and the mechanisms of such protection.

Methods: We have used a mouse model of infection, a multi-omic approach to characterize the microbiome (i.e. metagenomic, transcriptomic, metabolomic and culturomics) and in vitro models in order to identify commensal bacteria that restrict ARP intestinal colonization and to define possible mechanisms of protection.

Results: Metagenomic sequencing, in combination with culturomics and mouse models of infection identified specific commensal bacteria that restrict intestinal colonization of VRE (i.e. *Barnesiella*, *Alistipes*, Ruminococcaceae and *Olsenella* consortium) and MRE (i.e. *Lactobacillus*). In vivo transcriptomic and metabolomic analysis in combination with in vitro models suggest that the identified protective commensals prevent VRE and MRE gut colonization through competition for nutrient sources and production of inhibitory molecules, respectively. Our results may allow the design of alternative therapeutic approaches, based on the functional capacity of the gut microbiome, in order to prevent infections by pathogens resistant to nearly all available antibiotics.

O111 Microbe Atlas Project: analyze and compare your microbiome sample to a global reference set of a million microbiome samples

João Frederico Matias Rodrigues[1], Gregor Rot[1], Janko Tackmann[1], Sebastian Schmidt[2], Christian von Mering[1]

[1]*University of Zürich, Institute of Molecular Life Sciences, Zürich, Switzerland*
[2]*EMBL Heidelberg, Heidelberg, Germany*

Background: Large amounts of metagenomic sequence data have been collected from diverse microbial communities across the globe. The sheer amount of data, the differences between sequencing protocol and bioinformatics tools used, makes the quick and direct comparison of results across studies a cumbersome and difficult task.

Objectives: The Microbe Atlas Project (MAP) is a database, analysis platform and web resource providing access to more than one million analysed metagenomics samples. It aims to support and standardize microbial research by providing an online analysis pipeline and an integrated, consistently analysed set of reference metagenomics samples.

Methods: More than a million sequencing runs were obtained from the NCBI Sequence Read Archive and analysed using the MAPseq software. The closed reference 16S/18S ribosomal RNA reference used by MAPseq provides two advantages: 1) it enables consistent and comparable quantification of microbial taxa abundance across data originating from different sequencing approaches (shotgun, 16S/18S ribosomal RNA amplicon, and transcriptomic), and 2) enables the quantification of abundances for unclassified and poorly studied microbial taxa. Furthermore, the estimated microbial abundances were used to cluster the analysed samples into groups using microbial community similarity, enabling the identification of significant differential microbial abundances using the ZINBWAVE R package.

Results: The MAP database and web resource (http://www.microbeatlas.org/) enables researchers to upload and analyse their own sequence data, providing information on the typical environments of identified microbial taxa, as well as compare and identify significant differences in taxa abundances between their samples and typical microbial communities found in similar environments.

O112 Deciphering microbial interactions in a model microbiome

Manon Morin[1], Emily Pierce[1], Rachel Dutton[1;2]

[1]*University of California San Diego, Division of Biological Sciences, La Jolla, United States*
[2]*University of California San Diego, Center for Microbiome Innovation, Jacobs School of Engineering, La Jolla, United States*

Background: Microbial communities, also known as microbiomes, are ubiquitous and contribute to essential functions to sustain life on Earth. Microbial community structure and function rely on complex interactions whose underlying molecular mechanisms are poorly understood. Characterizing these interactions and associated mechanisms is key to uncover the principles governing microbial communities.

Objectives: Using a simple model microbiome from cheese rind, we aimed to design a novel strategy to investigate microbial interaction mechanisms. Also, relying on a bottom-up approach, we worked on understanding how interactions are structured in a microbiome and identify whether they are simple pairwise or more complex interactions.

Methods: To investigate these interactions in a simple microbiome, we introduced *E. coli* into an experimental community based on a cheese rind and identified the differences in *E. coli*'s genetic requirements for growth in interactive and non-interactive contexts (with increasing levels of complexity) using Random Barcode Transposon Sequencing (RB-TnSeq) and RNASeq.

Results: Genetic requirements varied among pairwise growth conditions and between pairwise and community conditions. Our analysis points to mechanisms by which growth conditions change as a result of increasing community complexity and suggests that growth within a community relies on a combination of pairwise and higher-order interactions. Our work provides a framework for using the model organism *E. coli* as a readout to investigate microbial interactions regardless of the genetic tractability of members of the studied ecosystem.

O113 Effects of maternal gut and breast milk microbiota on the infant gut microbiota composition, resistome and mobile genetic elements

Katariina Pärnänen[1], Antti Karkman[1;2;3], Jenni Hultman[1], Reetta Satokari[4], Christina Lyra[1], Johan Bengtsson-Palme[3;5], Joakim Larsson[2;3], Himanshu Kumar[6], Seppo Salminen[6], Samuli Rautava[7], Erika Isolauri[7], Marko Virta[1]

[1]*University of Helsinki, Department of Microbiology, Helsinki, Finland*
[2]*Department of Infectious Diseases, University of Gothenburg, Gothenburg, Sweden*
[3]*Center for Antibiotic Resistance research (CARe) at University of Gothenburg, Gothenburg, Sweden*
[4]*University of Helsinki, Immunobiology Research Program, Helsinki, Finland*
[5]*Department of Infectious Diseases, University of Gothenburg, Gothenburg,*
[6]*University of Turku, Functional Foods Forum, Faculty of medicine, Turku, Finland*
[7]*University of Turku, University of Turku and Turku University Hospital, Turku, Finland*

Background: The fecal bacterial microbiota of infants harbor diverse resistomes, distinct from those of adults. Antibiotic resistance genes are generally more abundant in infants, but their origins are largely unknown as they can be found even in infants without antibiotic exposure, although some of the genes are likely to be transmitted from the mother.

Objectives: Our objective was to determine the effects that maternal microbiota has on infant resistome and microbiome.

Methods: We studied the taxonomic composition, resistome and mobile genetic elements of the infant gut as well as maternal gut and breast milk microbiomes using shotgun metagenomic sequencing.

Results: Infant gut resistomes were largely shaped by bacterial phylogeny with *Escherichia coli* being highly correlated with resistance gene abundance. The resistance gene and mobile genetic element profiles of infants were more similar to their own mothers´ gut microbiota than to the microbiota of unrelated mothers. Interestingly, the same phenomenon was observed with the mobile genetic elements found in breastmilk. In addition, we observed that termination of breastfeeding and intrapartum antibiotic prophylactic treatment of mothers were linked to higher abundances of specific antibiotic resistance genes and mobile elements. We show that resistance genes and mobile genetic elements can be transferred from the mother to the infant gut directly via breast milk and indirectly from the maternal gut. Our results suggest that infants inherit the legacy of past antibiotic consumption of their mothers, but that the gut microbiota composition yet has a major impact on the overall resistance load.

O114 Escherichia coli prevents Salmonella Typhimurium infections after diet-shift-mediated microbiota perturbation

Markus Kreuzer[1], Sandra Y. Wotzka[1], Wolf-Dietrich Hardt[1]

[1]*Institute of Microbiology, D-BIOL, Zürich, Switzerland*

Background: An unperturbed microbiota blocks *Salmonella* gut colonization, the essential first step of the infection. In spite of this colonization resistance, *Salmonella* diarrhea remains prevalent in humans. Antibiotic-mediated microbiota perturbation can only explain a fraction of these infections. Thus, unknown factors may promote infections. Based on its known microbiota-modifying activity, we assessed shifts in food composition.

Objectives: We aimed to explore the role of short-term high-fat diet exposition and the mechanistic background of increased *S.* Typhimurium gut colonization. We wanted to assess the effect of increased bile salt levels as one driver of boosted *S.* Typhimurium colonization.

Methods: To explore the role of fat, we supplemented mice on maintenance diet with fat, oleic acid, a common fatty acid and major fat-degradation product, and bile salts. Subsequent infection with wild-type *S.* Typhimurium or mutants thereof helped to determine what factors are crucial for colonization.

Results: Shifting *E. coli*-free mice for 24h to high-fat or corresponding control diets boosted *S.* Typhimurium gut colonization and bloom-driven plasmid transfer. Bile salts compromise growth of most microbiota strains, while AcrAB/TolC-mediated efflux renders *S.* Typhimurium bile-resistant. We identify elevated bile-concentrations as one underlying principle and establish a mechanistic model of how the physiology of fat digestion perturbs colonization resistance. Competitive *E. coli* growth can protect from diet-shift-promoted *Salmonella* colonization. This establishes diet shifts and fat-elicited bile as potential risk factors for infection and resistance plasmid spread and identifies commensal *E. coli* spp. as a potent protective shield.

O115 Understanding how bacterial products from the microbiota enter the host, determining where they aggregate, and their influence over immune cells at these sites.

Christine Jordan[1], Tom Clarke[1]

[1]*Imperial College London, Medicine, United Kingdom*

Background: The microbiota is crucial for gut homeostasis by aiding in nutrient uptake, and protecting against pathogens. Recent evidence suggests the benefits provided by the microbiota are not restricted to the intestine but also extend to systemic sites. Systemic benefits are hypothesized to be mediated by bacterial products, derived from the microbiota, such as peptidoglycan and lipopolysaccharide, entering the bloodstream and acting as novel signalling molecules at distal sites.

Objectives: The precise way in which these microbial products enter the bloodstream remains largely unclear. We plan to provide mechanistic insight into how gut microbiota derived bacterial products enter the bloodstream, and the effect they exert on immune cells at systemic sites.

Methods: Using both *in vitro* and *in vivo* models we can examine bacterial product translocation acorss the intestinal epithelium. Using fluorescently labbelled bacterial products has allowed us to establish an *in vivo* tracking model to determine in which organs these products disseminate.

Results: Our data suggest bacterial products can cross the intestinal epithelium, and routes across may vary between different bacterial products. We find that host processing of cell wall molecules, by host antimicrobial lysozyme, promotes their translocation across the epithelium. Once in the bloodstream our preliminary data provide support that the liver plays a role in clearing bacterial products from the blood, as here we see a reservoir of peptidoglycan. This increased dissemination of cell wall molecules additionally enhances resistance to pulmonary infection. Therefore lysozyme treatment enhances bacterial product migration and increases host protection against systemic pathogens.

O116 Isolation and culture of Faecalibacterium strains from fecal samples using polyclonal antibodies and cell sorting in anaerobic conditions

Samuel Bellais[1], Maryne Ania[1], Melanie Nehlich[1], Jan Baijer[2], Vincent Thomas[1]

[1]*Bioaster, Paris, France*
[2]*Commissariat à l'Energie Atomique, Fontenay-aux-Roses, France*

Background: There is a growing interest in using gut commensal bacterial species as 'next generation' probiotics. However, this approach is still hampered by the fact that there is limited number or even no strains available for specific species that are usually difficult to cultivate.

Objectives: Our objective was to adapt flow cytometry and cell sorting to be able to detect, sort and cultivate new strains of Extremely Oxygen Sensitive (EOS) species from fecal material.

Methods: The BD Influx® cell sorter was equipped with a glovebox that covers the sorting area, and that is fed with nitrogen to deplete oxygen in the enclosure. Several non-specific staining methods including Wheat Germ Agglutinin (WGA), Vancomycin BODIPY™ and LIVE/DEAD BacLight were evaluated with three different strains of the EOS species *Faecalibacterium prausnitzii*. In parallel, we generated polyclonal antibodies directed against this species by immunizing rabbits with LPxTG proteins or heat-inactivated bacteria.

Results: Anaerobic conditions were maintained during the full process, resulting in only minor viability loss during sorting and culture of un-stained *F. prausnitzii* reference strains. In addition, staining solutions did not severely impact bacterial viability while allowing discrimination between strains. Efficient detection was achieved using polyclonal antibodies directed against heat-fixed bacteria, whereas antibodies generated using LPxTG proteins presented only limited efficacy. Finally, we were able to detect, isolate and cultivate a variety of *F. prausnitzii* strains from healthy volunteer's fecal samples using WGA staining and antibodies. These strains present markedly different phenotypes, thus confirming the heterogeneity of the species.

O117 Spatially discrete micro-niches govern root microbiome assembly

Bridget O'Banion[1], Thomas Hyde[1], Lindsey O'Neal[2], Gladys Alexandre[2], Sarah Lebeis[1]

[1]*University of Tennessee-Knoxville, Department of Microbiology, Knoxville, United States*
[2]*University of Tennessee-Knoxville, Department of Biochemistry & Cellular and Molecular Biology*

Background: Plant rhizospheres host complex microbial communities. Two major factors influencing the root-associated microbiome are exudate composition and microbe-microbe interactions. Mechanistic understanding of how chemotactic localization to root exudates and subsequent competitive interactions combine to dictate microbiome diversity remains enigmatic. Many plant microbiome studies overlook either the biotic complexity or the spatial heterogeneity of the root system, despite evidence that these factors strongly contribute to the evolution and maintenance of biodiversity across ecosystems.

Objectives: We aim to identify specific microbial factors contributing to plant-microbe and microbe-microbe interactions at different root locations. We use a range of biotic complexity (single strain to synthetic community) and spatial heterogeneity (*in vitro* to *in planta*) to gain both a mechanistic and holistic understanding of how individual strain phenotypes scale-up to higher-order community dynamics.

Methods: We first define the fundamental niche of individual bacterial strains in the context of the root by investigating exudate utilization, chemotactic localization, and bulk tissue colonization. We then compare results from *in vitro* and *in planta* bipartite interaction assays to *in silico* primary and secondary metabolic predictions to classify competitive interactions between bacterial strains. Lastly, we assess the impact of spatial organization and competition on rhizosphere microbiome establishment using a variety of partially overlapping synthetic bacterial communities.

Results: We find that individual bacterial strains have distinct phenotypes in the context of plant roots and these behaviors can be explained by genetic factors. Communities with varying degrees of metabolic overlap and antagonism have different spatial organization patterns and long-term biodiversity levels.

O118 Unstable Antibiotic Resistance

Dan I. Andersson[1]

[1]*Uppsala University, Medical Biochemistry and Microbiology, Uppsala, Sweden*

Background: Bacterial populations often show heterogeneity in their levels of phenotypic antibiotic resistance, and in a majority of cases the causative genetic mechanisms underlying the heterogeneity remain unknown. One example of a clinically relevant heterogeneity is heteroresistance (HR) where small subpopulations of a bacterial isolate can grow at antibiotic concentrations well above (>8-fold) the MIC of the main population.

Objectives: To determine the prevalence and underlying mechanisms of HR in *Salmonella enterica, Escherichia coli, Klebsiella pneumoniae* and *Acinetobacter baumanii*.

Methods: Population analysis profiling and E-tests were used to screen for HR. Whole genome sequencing, qPCR, deletion- and overexpression-analyses were used to define the genetic mechanisms underlying HR. Mathematical modeling were used to explore the conditions under which HR can cause treatment failure.

Results: A screen of HR in 41 clinical isolates of several Gram-negative bacterial species against 28 different antibiotics showed that 27.4% of the 766 species-antibiotic combinations tested exhibited HR and in most cases the observed HR was unstable. The mechanism of HR involved unstable tandem gene amplification of various resistance genes (mainly antibiotic modifying enzymes). Mathematical modeling showed that in the parameter range estimated in this study HR can result in the failure of antibiotic treatment of infections with bacteria that are classified as antibiotic susceptible. The high prevalence of HR with the potential to cause treatment failure highlights the limitations of MICs as a unique criterion for susceptibility determinations. These results call for development of facile and rapid protocols to identify HR in pathogens.

O119 Predicting and preventing the evolution of antibiotic resistance across Staphylococcus aureus

Craig Maclean[1]

[1]*Department of Zoology, University of Oxford, United Kingdom*

Background: The rise of antibiotic resistance in many pathogens has been driven by the spread of a few dominant epidemic lineages. Are successful lineages 'lucky', or do some strains have an increased ability to evolve antibiotic resistance? If so, which genes/pathway accelerate the evolution of resistance?

Objectives: Our goal was to test for variation in the ability to evolve resistance between strains of *S.aureus*, and to identify the genes underlying this variation.

Methods: We challenged 12 replicate cultures of 263 clinical isolates of *S.aureus* with ciprofloxacin under controlled conditions. Following this, we used extensive whole genome resequencing of evolved isolates, RNA-seq transcriptomic profiling and GWAS to identify genes that accelerate the evolution of antibiotic resistance. Finally, we manipulated the expression of the *NorA* efflux pump in the RN4220 model strain to test the role that this gene plays in evolvability.

Results: The ability to evolve resistance to ciprofloxacin varied profoundly between strains of *S.aureus.* This variation did not arise because strains followed different genetic routes to evolving ciprofloxacin resistance. Instead, both high and low evolvability strains evolved ciprofloxacin resistance by classic target modification mutations. We found that intrinsic variation in the expression of the *NorA* efflux pump was associated with high evolvability across clinical isolates, and we confirmed this association by manipulating *NorA* expression in the RN4220 strain. Importantly, combining ciprofloxacin with the *NorA* inhibitor reserpine allowed us to prevent the evolution of resistance. Our study shows that it is possible to predict and prevent the emergence of resistance.

O120 Using higher-order drug combinations and emergent properties to combat multi-drug resistant pathogens

Pamela Yeh[1]

[1]*Univ Calif Los Angeles, Ecology and Evolution, United States*

Background: Drug treatments increasingly rely on using multiple drugs in combination. Yet there is limited understanding of how bacterial populations are impacted by higher-order combinations — combinations with three or more drugs.

Objectives: In this talk I will explain new conceptual and experimental work to examine higher-order interactions among antibiotics in bacterial populations, and discuss implications to combat multi-drug resistant bacteria.

Methods: We use high-throughput experimental techniques as well as the development of novel conceptual mathematical models.

Results: We find that higher-order drug combinations have increased percentages of interactions, and increased anatgaonistic interactions.

O121 Genomics-based epistasis analysis reveals fitness costs hampering the evolution of MRSA

Ruth Massey[1]

[1]*University of Bristol, School of Celular and Molecular Medicine, Bristol, United Kingdom*

Background: Fitness costs imposed on bacteria by antibiotic resistance mechanisms are believed to hamper their dissemination. The scale of these costs is highly variable. Some, including resistance of *Staphylococcus aureus* to the clinically important antibiotic mupirocin, have been reported as being cost-free, suggesting there are few barriers preventing their global spread. However, this is not supported by surveillance data which indicate that this resistance mechanism is relatively unsuccessful.

Methods: Epistasis analysis on two collections of MRSA provides an explanation for this discord, where the mupirocin resistance-conferring mutation of the *ileS* gene affects the levels of toxins produced by *S. aureus* when combined with polymorphisms at other loci. Proteomic analysis demonstrates that the activity of the secretory apparatus of the PSM toxin family is affected by mupirocin resistance. As an energetically costly activity, this reduction in toxicity masks the fitness costs associated with this resistance mutation, a cost that becomes apparent when toxin production becomes necessary.

Conclusion: This hidden fitness cost provides a likely explanation for why this mupirocin-resistance mechanism is not more prevalent, given the widespread use of this antibiotic. With dwindling pools of antibiotics available for use, information on the fitness consequences of the acquisition of resistance may need to be considered when designing antibiotic prescribing policies. However, this study suggests there are levels of depth that we do not understand, and that holistic, surveillance and functional genomics approaches are required to gain this crucial information.

O122 Basic microbiology, chemistry and synthetic biotechnology to identify and characterize antibiotics from microbes

Rolf Müller[1]

[1]*Helmholtz Institut für Pharmazeutische Forschung Saarland, Germany*

Background: According to the World Health Organization

- Antimicrobial resistance (AMR) threatens the effective prevention and treatment of an ever-increasing range of infections caused by bacteria, parasites, viruses and fungi.
- Antibiotic resistance is one of the biggest threats to global health, food security, and development today.

Objectives: The presentation aims to provide knowledge about current projects and ideas aiming to overcome antibiotic resistance by generating novel natural product based antimicrobials exhibiting innovative mode of action.

Methods:

- Novel Natural Products, their resources and production processes
- Mode of action analysis of natural products

Results: Amongst the well-established bacterial producers myxobacteria have a great track record for the discovery of entirely new natural product scaffolds exhibiting promsing bioactivities[1]. This is at least in part due to the fact that they have been much less studied in the past in comparison to other traditional sources such as actinomycetes and bacilli. Nevertheless, the issue of rediscovery is a major hurdle for myxobacterial extracts as well. I will disucss recent results from our efforts to culture previously uncultured myxobacteria and to connect phylogentically distant clades to novel metabolites by metabolome and genome mining[2]. Examples of novel and genetically engineered natural products in preclinical development as broad spectrum antibiotics exhibiting novel mode of action(s) will be shown [3,4,5,6] In addition, I will show examples of heterologous expression of myxobacterial compounds yielding producer strains making production of lead compounds for pharmaceutical development feasible[7].

O123 Genetic and pharmacological inactivation of D-alanylation of (lipo-)teichoic acids re-sensitizes pathogenic enterococci to beta-lactams

Delphine Coupri[1], Aurélie Budin-Verneuil[1], Axel Hartke[1], Abdellah Benachour[1], Thierry Lequeux[2], Emmanuel Pfund[2], Nicolas Verneuil[1]

[1]Normandie Univ, UNICAEN, U2RM, 14000 Caen, France
[2]Normandie Univ, LCMT, ENSICAEN, UNICAEN, CNRS, 14000 Caen, France

Background: The dramatic increase of drug resistance in major human pathogens is a serious global problem. Enterococci intrinsically resistant to cephalosporins are part of these critical pathogens for which new treatment strategies are urgently needed.

Objectives: We explored if the D-alanylation system of teichoic acids (TAs) could be a promissing drugable target to fight infections caused by drug resistant enterococci. TAs are cell wall polymers decorated with D-alanine. Four enzymes (DltA to DltD) encoded by the dlt-operon are necessary for the addition of D-alanine.

Methods: Growth and survival of enterococci strains were analysed after treatment with several clinically relevant antibiotics individually and in combinations . An inhibitor of DltA was synthesized and tested in vitro and in an infection model based on the larvae of the insect Galleria mellonella infected with *E. faecalis* and *E. faecium*.

Results: Absence of D-alanylation does not impact the MICs towards the tested antibiotics. Furthermore, most cephalosporins used in monotherapy had no impact on the parental strain survival but were slightly lethal for the dltA mutant of *E. faecalis*. Addition of a very low concentration of amoxicillin was synergistic under these conditions. The most spectacular effect was obtained with a combination of cefotaxime (1µg/mL) and amoxicillin (0.03 µg/mL). In the presence of the synthesized inhibitor, the wild-type strain was as sensitive to this combinatory treatment as the dltA mutant. Moreover, we provide the proof-of-concept that pharmacological inactivation of DltA with this combination is also efficient to protect the larvae against lethal infections by enterococci.

O124 New type of synthetic antimicrobials targeting respiratory sodium pump in gram-negative pathogens

Pavel Dibrov [1], Elena Dibrov[2], Craig Resch[2], Ryan Ramjiawan[1], Kim A. O'Hara[2], David Nelson[2], Jordan Nelson[2], Grant N. Pierce[2]

[1]*University of Manitoba, Microbiology, Winnipeg, Canada*
[2]*Albrechtsen Research Centre, Institute of Cardiovascular Sciences, Winnipeg, Canada*

Background: The unfolding crisis in antibiotic development and administering was precipitated not only by global misuse of broad-spectrum antibiotics and resulting proliferation of multi-drug resistant (MDR) strains, but also by a general strategy in antibiotic design, when a very limited set of prokaryotic enzymes and metabolic pathways was ever targeted. The situation calls for urgent intensification of the search for non-traditional antimicrobial targets.

Objectives: Na^+-translocating NADH:ubiquinone oxidoreductase (Na^+-NQR) acts as a major respiratory Na^+ pump in diverse Gram-negative pathogens including *Chlamydia trachomatis, Legionella pneumophila, Vibrio cholerae, Neisseria gonorrhoeae* and many others. Inhibition of Na^+-NQR was expected to arrest bacterial energization and proliferation, and ultimately disrupt the infectious process. Importantly, Na^+-NQR inhibition would indirectly affect MDR pumps thus re-sensibilizing pathogens to conventional antibiotics.

Methods: A new furanone inhibitor of Na^+-NQR, PEG-2S, was designed and assayed for its anti-microbial activity in both liquid culture and two-component cell culture model of infection. PEG-2S was further used as a molecular platform for the design of a new line of Na^+-NQR inhibitors.

Results: PEG-2S and its derivatives inhibited the Na^+-NQR activity in sub-bacterial membrane vesicles isolated from *V. cholerae* at nanomolar concentrations. These compounds also inhibited intracellular proliferation of *Chl. trachomatis* with a half-minimal concentration in the sub-micromolar range without affecting the viability of mammalian cells or selected species representing benign intestinal microflora. Further, PEG-2S-based inhibitors effectively arrested growth of free-living *L. pneumophila* in liquid cultures. Taken together, these results demonstrate that Na^+-NQR is highly attractive as a target for prospective narrowly targeted antimicrobial remedies.

O125 Targeted protein antibiotic therapy for Pseudomonas aeruginosa sepsis

Anne Six[1], Khedidja Mosbahi[1], Madhuri Barge[1], Thomas Evans[1], Daniel Walker[1]

[1]*University of Glasgow, Institute of Infection, Immunity and Inflammation, Glasgow, United Kingdom*

Background: In the past few years, there has been a worldwide rise of bacterial antibiotic resistance. New therapeutic agents are urgently needed to treat multi-resistant and chronic infections. Amongst those threats, *Pseudomonas aeruginosa* is a Gram-negative pathogen frequently resistant to conventional antibiotics. It is responsible for severe hospital-acquired blood and lung infections as well as being an important pathogen responsible of chronic lung infections in patients suffering from cystic fibrosis. A promising strategy is the development of bacteriocins, such as the *P. aeruginosa* expressed pyocins, as species-specific protein antibiotics. Previous work on pyocins has demonstrated their activity against a pathogenic strain of *P. aeruginosa* in a murine model of acute lung infection on post-infection administration (McCaughey *et al*. 2016).

Objectives: Our current goal is to further our understanding of the mechanism of pyocin action and demonstrate their efficacy for the treatment of *P. aeruginosa* sepsis.

Methods: For this purpose, we have developed a *P. aeruginosa* sepsis murine model of infection. Pyocin distribution in the host was followed using quantitative and qualitative methods. We then assessed the efficacy and impact of pyocin treatment of infected mice in this model by following survival, colony counts and cytokine quantification.

Results: We show that pyocins S5 and AP41 are stable and active *in vivo* and a single injection of pyocin post-infection improves survival rates compared to sham-treated mice. Overall, the results obtained illustrate the potential of pyocins to be developed into therapeutics for the treatment of *P. aeruginosa* infections.

O126 Engineered toxin-intein antimicrobials can selectively target and kill antibiotic-resistant bacteria in mixed population

López-Igual Rocio[1], Bernal-Bayard Joaqin[2], Rodríguez-Patón Alfonso[3], Ghigo Jean-Marc[2], Didier Mazel[1]

[1]*Institut Pasteur, Unié Plasticité du Génome Bactérien, Paris, France*
[2]*Institut Pasteur, Unié Génétique des Biofilms, Paris, France*
[3]*Universidad Politécnica de Madrid, Departamento de Inteligencia Artificial, Madrid, Spain*

Background: Antibiotic resistance is becoming a major concern worldwide. Targeted killing of pathogenic bacteria without harming beneficial members of host microbiota holds promise as a strategy to cure disease, and limit both antimicrobial-related dysbiosis and development of antimicrobial resistance.

Objectives: We constructed a synthetic system, which can perform killing of specific pathogenic bacteria within mixed populations, such as gut microbiota**.**

Methods: Our system is based on the coupling of artificially split toxins (non-functional) with split intein technology. Inteins are protein sequences embedded inside a *host protein* from where they can be self excised by protein splicing. Naturally, inteins exist also as split modules. We engineer toxins from type II toxin-antitoxin modules, that are split by inteins and deliver them by conjugation into a mixed population of bacteria. Our toxin-intein antimicrobial is only activated in bacteria that harbor specific transcription factors.

Results: We apply our antimicrobial to specifically target and kill antibiotic resistant Vibrio cholerae in complex populations gathering various bacterial species. We found that 100% of antibiotic resistant V. cholerae receiving the plasmid were killed. Escape mutants were extremely rare (10^{-6}-10^{-8}). We demonstrate that conjugation and specific killing of targeted bacteria is functional in the microbiota of zebrafish and crustacean larvae[1], which are natural hosts for *Vibrio* spp. Toxins split with inteins could form the basis of a range of precision antimicrobials which would kill both Gram – and Gram + pathogens.

[1], Lopez-Igual R, et al**.** Engineering synthetic toxin-intein weapons as specific antimicrobials. *Nature Biotechnology* (2019) Apr 15. doi: 10.1038/s41587-019-0105-3.

O127 Biosynthetic engineering for the development of atypical tetracycline antibiotics

Hrvoje Petkovic[1]

[1]*University of Ljubljana, Biotechnical Faculty, Ljubljana, Slovenia*

Background: There is an urgent need to bring into the clinic as quickly as possible new antibiotics from novel antibiotic classes that bypass current resistance mechanisms, preferably with new mode of action. In drug development, a number of approaches, such as screening natural product libraries and attempts to develop chemically synthesized antibiotics based on novel targets showed that the identification of promising anti-infective lead structures is a rare event.

Objectives: One rather under-utilized alternative is the re-assessment of unexploited structural scaffolds with already proven antibacterial potency and resistance-breaking properties, and to advance these through the development process using cutting-edge biosynthetic engineering and semi-synthetic approaches.

Methods: Natural tetracycline antibiotics were the first major class of therapeutics to earn the distinction of “broad-spectrum antibiotics” and they have been used since the 1940s against a wide range of both Gram-negative and Gram-positive pathogens, mycoplasmas, intracellular chlamydiae, rickettsias and protozoan parasites. In collaboration between the Biotechnical faculty of the University of Ljubljana, Acies Bio Ltd. (Slovenia) and the Helmholtz Institute for Pharmaceutical Research, (Saarbrücken, Germany), we have applied biosynthetic engineering efforts to develop new tetracycline analogues.

Results: By applying biosynthetic engineering approaches, we generated diverse tetracycline analogues. These display a currently unprecedented mode of action and are presently at the lead optimization stage with potent activity even against multidrug-resistant Gram-negative pathogens belonging to the ESKAPE group. Biosynthetic efforts applied in the scope of his project will be presented.

O128 Analogue of antimicrobial peptide LL III - a potent agent inhibiting biofilm formation of Candida albicans

Petra Kašparová[1;2], Eva Vaňková[1;2], Olga Maťátková[2], Václav Čeřovský[1]

[1]*Institute of Organic Chemistry and Biochemistry AS CR, Antimicrobial peptides, Praha 6 - Dejvice, Czech Republic*
[2]*University of Chemistry and Technology, Prague, Faculty of Food and Biochemical Technology, Praha 6 - Dejvice, Czech Republic*

Background: Antimicrobial peptides (AMPs) are considered as a possible new class of compounds that could be used as a supplement to, or substitute for, conventional antibiotics in the fight against drug-resistant microbes. Because their mechanism of action differs from antibiotics, microbial resistance to AMPs is less likely to be developed. LL III/43 is a structurally modified analogue of naturally occurring α-helical pentadecapeptide lasioglossin (LL-III) originally isolated from the venom of the eusocial bee *Lasioglossum laticeps*.

Objectives: This study focuses on the effect of LL III/43 as well as commercial antimicrobials - antifungal azoles on biofilm formation of *Candida albicans* and on production of its virulent factors. *C. albicans* is a severe opportunistic pathogen. Its potent ability to form biofilm as well as the considerable production factors of virulence (e.g. proteases, lipases, and hemolysins) significantly contributes to the prevalence of *C. albicans* infections.

Methods: The effect of LL-III/43 and antifungal azoles (clotrimazole, fluconazole, voriconazole) on the formation of *C. albicans* biofilm was studied based on the evaluation of the metabolic activity of cells persisting inside the biofilm using resazurin viability assay. The virulent factors were determined using agar plate methods or colorimetric assays. Hydrophobicity of cellular surface was determined using MATH assay.

Results: Unlike antifungal azoles, the LL III/43 effectively inhibited biofilm formation of all tested clinical isolates of *C. albicans*. This peptide also decreased the hydrophobicity of *C. albicans* cellular surface and it had synergistic effect when used in combination with azoles against biofilm cells.

O129 Synthetic and diverse - microbiology on duty in industry

Michael Sauer[1;2;3]

[1]*CD Laboratory for Biotechnology of Glycerol, Vienna, Austria*
[2]*BOKU - University of Natural Resources and Life Sciences, Department of Biotechnology, Vienna, Austria*
[3]*Austrian Centre of Industrial Biology, Wien, Austria*

Background: In our societies quest to mitigate greenhouse gas emissions and petroleum use, industrial microbiology plays a key-role for the provision of processes for fuel and chemical production from renewable resources. Clearly, the microorganism is in the centre of the process and care should be taken for its choice. Industrial production conditions are generally very harsh for the microorganism. Nevertheless, the host cells should be very efficient, which opens a vast area of conflict for the industrial microbiologist. Synthetic biology and metabolic engineering provide optimal tools for the rational design of biocatalysts. However, biodiversity is a major resource which should be tapped first. Nature solved many problems, which we face in industrial context. However, all too often the rich source of natural diversity is neglected in favour of "pet" or model organisms. I propose that the fastest and most reliable path to efficient and economically viable microbial production processes uses both – natural diversity and synthetic biology.

Objectives: The importance of biodiversity will be exemplified with processes for 1,3-propanediol and butanol production.

Methods: Proper bioprocess engineering and synthetic approaches must go hand in hand to develop an economically viable production process.

Results: 1,3-propanediol production with *Lactobacillus diolivorans* from crude glycerol has been developed as an economically viable process which is being industrialized. A titre exceeding 90 g/L and a yield based on glycerol of over 90% has been obtained.

Clostridia and a combined process of *Serratia marcescens* and *L. diolivorans* are explored for 1-butanol and 2-butanol production, respectively.

O130 Filamentous fungal cell factories for human welfare and a sustainable bioeconomy: Prospects and challenges

Vera Meyer[1]

[1]*Technische Universität Berlin, Applied and Molecular Microbiology, Berlin, Germany*

Fungal biotechnology is currently undergoing a renaissance with important implications for its role as platform technology for the sustainable production of products, goods and drugs. Allied to this are the recent advances in fungal systems and synthetic biology. Systems-level understanding of the filamentous lifestyle and development of sophisticated synthetic biological tools for controlled manipulation of fungal genomes now allow rational strain development programs based on data-driven decision making. In my talk, I will focus on *Aspergillus niger* to summarize recent insights into the multifaceted and dynamic relationship between filamentous growth and product formation from genetic, metabolic, subcellular, macromorphological and process engineering perspectives. Current progress and knowledge gaps with regards to mechanistic understanding of product formation and export from the fungal cell are discussed. I will highlight possible strategies for unlocking lead genes for rational strain optimizations based on -omics data, and discuss how targeted genetic manipulation of these candidates can be used to optimize *A. niger* for improved production of enzymes and natural products.

O131 Interspecific hybridization in beer yeast: going beyond Saccharomyces cerevisiae diversity

Jan Steensels[1;2], Brigida Gallone[1;2;3;4], Stijn Mertens[1;2], Jonathan L. Gordon[1;2], Ben Souffriau[5], Philippe Malcorps[5], Luk Daenen[5], Franz Meussdoerffer[6], Mathias Hutzler[6], Troels Prahl[7], Guy Baele[8], Steven Maere[3;4], Kevin Verstrepen[1;2]

[1]*VIB, Center for Microbiology, Leuven, Belgium*
[2]*KU Leuven, Microbial and molecular systems, Leuven,*
[3]*VIB, Center for plant systems biology, Ghent, Belgium*
[4]*Ghent University, Departement of plant biotechnology and bioinformatics, Ghent, Belgium*
[5]*AB-InBev SA/NV, Leuven, Belgium*
[6]*TU Munich, Research Center Weihenstephan for brewing and food quality, Freising, Germany*
[7]*White Labs Inc., San Diego, United States*
[8]*KU Leuven - Rega Institute, Departement of microbiology and immunology, Leuven, Belgium*

Background: Yeasts have been associated with food and beverage fermentations for thousands of years, and drive many industrial fermentations today. The industrial yeast species par excellence is *Saccharomyces cerevisiae*, more commonly known as baker's or brewer's yeast. In a process called 'domestication', it gradually adapted to man-made fermentation environments, leading to the immense biodiversity that exists today.

Interestingly however, it is now becoming increasingly clear that *S. cerevisiae* is not the only *Saccharomyces* species that contributes to industrial fermentation processes. Non-*cerevisiae* species usually do not occur as such in the fermentation environment, but are present as interspecific hybrids.

Objectives: While interspecific hybrids have been reported in all kingdoms of life, many questions on the ecological circumstances and evolutionary aftermath of interspecific hybridisation are still unanswered. Using industrial yeast as a model, we investigated the cause and consequence of interspecific hybridization, and its role in evolution and domestication.

Methods: Using sequencing and large-scale phenotyping, we have characterized a large set of yeasts originating from the beer/brewery environment.

Results: We describe how interspecific hybridisation provides an important evolutionary route allowing swift adaptation to novel environments. Several hybrids between *Saccharomyces* species originated in industrial environments, and post-hybridisation evolution led to sub-specialisation and adaptation to specific beer styles, a process accompanied by extensive chimerisation between subgenomes. Using molecular dating, we mapped evolutionary events in a historical perspective to gain insight into the driving forces behind yeast hybrid domestication.

O132 Sustainable production of chemicals, fuels and animal feed from C1 gases

Nigel Minton[1], Christian Arenas[1], Jon Baker[1], Kamran Jawed[1], James Millard[1], Giorgia Tibaldero [1], Katalin Kovács[1]

[1]*University of Nottingham, BBSRC/EPSRC Synthetic Biology Research Centre (SBRC), School of Life Sciences, Centre for Biomolecular Sciences, United Kingdom*

The future sustainable production of chemicals and fuels from non-petrochemical resources and the reduction of greenhouse gas emissions are two of the greatest challenges facing society. Developing economic processes based on lignocellulosic (biomass) as a feedstock is, however, proving extremely challenging. There is, however, an exciting low-cost alternative, and that is to directly capture carbon, by harnessing the ability of autotrophic bacteria to 'eat' single carbon GHG gases such as CO_2. The gas is injected into the liquid medium of fermentation vessels where it is consumed by the metabolically engineered bacteria [*Current Opinions Biotechnology*. 2018; **50**:174-181] and converted into the chemicals and fuels we need. Fortunately, such gases are an abundant resource, and may be derived from non-food sources such as waste gases from industry as well as 'synthesis gas' produced from the gasification (heating) of non-food biomass and domestic/ agricultural wastes.

The ENGICOIN consortium comprises 12 academic and industrial partners from across Europe (Italy, Holland, Germany, Belgium, Austria, Spain and the UK) and aims to devise microbial-based processes that will consume the CO_2 released by the anaerobic digestion of biomass waste, from landfill and from a water treatment plant. The chassis (and products) being developed are a phototrophic cyanbacteria (polylactic acid), the anaerobic acetogen *Acetobacterium woodii* (acetone) and the chemolithotrophic *Cupriavidus necator* (bio-degradable *polyhydroxyalkanoates* polymers).

By using non-food, waste gas as a feedstock, competition with food and land resources is avoided while at the same time providing benefits to the environment and society through a reduction in GHG emissions.

O133 Fermentation technology: how to transform agricultural by-products into valuable food ingredients

Rossana Coda[1]

[1]*University of Helsinki, Department of Food and Nutrition, Helsinki, Finland*

Background: Sustainability science suggests to increase the use of agricultural side streams for food purposes and to rely on accessible, domestic resources. In this context, microbial production systems can contribute to a more sustainable and efficient agro-food chain. The major part of agricultural by-products have a valuable nutritional composition. However, as such, they often possess challenging technological properties and perform poorly in food applications thus remaining under-utilised for their potential. Bioprocessing is a feasible option to enhance the overall quality of technologically challenging matrices such as brewers´spent grain, cereal bran and others.

Objectives: Fermentation with lactic acid bacteria can enhance the functionality of the raw material e.g. with the synthesis of exopolysachcarides (EPS) and improvement of nutritional properties. Identification of the most suitable strains and fermentation conditions allow an optimal synthesis of EPS and upgrade the quality of the matrix.

Methods: The performance of different lactic acid bacteria strains was followed through growth and metabolite formation, transcription analysis of exopolysaccharides biosynthethic genes, dextran formation and overall quality of the fermented raw material.

Results: The synthesis of EPS in different substrates depends on specific strain features and environmental conditions. Selection of strains and proper fermentation parameters is a pre-requisite to optimize the functionality of a raw material and to tailor its properties in the most desired way. Several investigations demonstrated the efficiency of lactic acid bacteria fermentation to turn agricultural waste into a food ingredient with desirable features e.g. rich in EPS and having good nutritional, technological and sensory properties.

O134 Lactic acid bacteria fermentation as a tool to improve the antioxidant properties of brewers' spent grain

Michela Verni[1], Daniela Pinto[2], Vito Verardo[3], Rossana Coda[4], Carlo Giuseppe Rizzello[1]

[1]*University of Bari Aldo Moro, Department of Soil, Plant and Food Science, Bari, Italy*
[2]*Giuliani Spa, Milano, Italy*
[3]*University of Granada, Department of Nutrition and Food Science, Granada, Spain*
[4]*University of Helsinki, Department of Food and Environmental Science, Helsinki, Finland*

Background: Brewers' spent grain (BSG), the main by-product of the brewing industry, is mainly constituted by fibers and protein. It is also rich in polyphenolic compounds, the majority of which is bound to cell wall material. Despite its functional potential, BSG is used as low value feed or discarded as waste.

Objectives: Aiming at promoting the use of BSG in functional food industry, a biotechnological protocol to enhance the antioxidant activity was set-up. Selected lactic acid bacteria (LAB) and food grade enzymes were used.

Methods: Thirty-two LAB were screened for the ability to grow in BSG and improve the antioxidant activity of the fermented matrix. A biotechnological protocol for BSG fermentation using selected LAB strains was set-up and optimized. The addition of cell-wall-degrading enzymes was also evaluated. *In vitro* assays and tests on murine fibroblasts cultures after induced oxidative stress were performed. A characterization of the phenolic profile on the bioprocessed matrix was performed by RP-HPLC-MS.

Results: All strains grew 1-2 log cycles during fermentation, but only *Lactobacillus plantarum* PU1, *Leuconostoc citreum* PRO17 and *Lactobacillus brevis* H46 caused relevant increases of the scavenging activity towards DPPH and ABTS radicals. The xylanase treatment slightly improved the antioxidant activity, especially when the addition was carried out before fermentation. The fermented BSG extracts showed protective effect on fibroblasts subjected to oxidative stress, as determined by the survival rate and the intracellular antioxidant activity (with MTT and DCFH-DA assays, respectively).

O134a Studies on the Effect of Lactic Acid Bacteria Consortium Fermentation on some Vitamin and Mineral Content of Soybean Flour

Alloysius Chibuike Ogodo [1], Ositadinma Chinyere Ugbogu [2], Hope Chukwuemeka Okereke [2]

[1]Department of Microbiology, Faculty of Pure and Applied Sciences, Federal University bWukari, P.M.B. 1020 Wukari, Taraba State, Nigeria.

[2]Department of Microbiology, Faculty of Biological and Physical Sciences, Abia State University Uturu, P.M.B 2000 Uturu, Abia State, Nigeria.

Background: Soybean is a nutritious crop and one of the world's legumes which are rich in vitamins and minerals.

Objectives: This study investigated the effect of fermentation using lactic acid bacteria (LAB)-consortium on the vitamin and mineral content of soybean flour.

Methods: Soybean seeds were processed into flour, fermented spontaneously and with LAB consortium (Lactobacillus nantensis LP33, Lactobacillus plantarum WCFS1, Lactobacillus fermentum CIP102980, Lactobacillus rhamnosus GG ATCC53/03, Lactobacillus reuteri DSM20016, Lactobacillus brevis ATCC14869, Pediococcus acidilactici DSM20284) previously isolated from maize and sorghum to determine their effect on the mineral and vitamin content of the flour using standard protocols at 12 h interval.

Results: The result showed a significant ($p<0.05$) increase in vitamin A, E, C, B1 and B2 as fermentation time increases in all fermentation set-ups. The mineral content increased significantly ($p<0.05$) as fermentation time increased. The increase ranged from 2.29 ± 0.16mg/100g to 4.22 ± 0.21mg/100g (calcium), 699.12 ± 0.99mg/100g to 724.10 ± 0.10mg/100g (phosphorus), and 2.03 ± 0.03mg/100g to 3.69 ± 0.12mg/100g (sodium) while iron increased from 16.00 ± 0.15mg/100g to 20.89 ± 0.03mg/100g. The increases were more in the LAB-consortia fermented samples than the spontaneous fermentation. This is an indication of the potentials of LAB consortium in improving the mineral and vitamin content of soybean flour which can be employed by food industries in food fortification.

O135 Maize milling by-products: from food wastes to nutritive improvers through lactic acid fermentation

Erica Pontonio[1], Cinzia Dingeo[1], Marco Gobbetti[2], Carlo Giuseppe Rizzello[1]

[1]*University of Bari, Department of Soil, Plant and Food Science, Bari, Italy*
[2]*Free University of Bozen, Faculty of Science and Technology, Italy*

Background: Although recognized as important sources of functional compounds, milling by-products are often removed from the cereal kernel prior milling process. Indeed, the high presence of fiber in bran and the co-presence of lipids and lipase in germ are often considered as downsides for breadmaking.

Objectives: In this work, the effects of lactic acid fermentation on the biochemical and nutritional features as well as the stability of the maize milling by-products were investigated.

Methods: *Lactobacillus plantarum* T6B10 and *Weissella confusa* BAN8 were used as selected starters to ferment maize milling by-products mixtures made with heat-treated or raw germ and bran. Fermented milling by-products mixtures were also used to fortify wheat bread.

Results: Lactic acid bacteria metabolisms improved the free amino acids and peptides concentrations and the antioxidant activity and caused a relevant phytic acid degradation. Moreover, fermentation allowed a marked decrease of the lipase activity, stabilizing the matrix by preventing oxidative processes. The use of fermented by-products as ingredients improved the nutritional, textural and sensory properties of wheat bread. Fortified breads (containing 25% of fermented by-products) were characterized by a concentration in dietary fiber and proteins of *ca.* 11% and 13% of dry matter, respectively. The addition of pre-fermented by-products to bread led to protein digestibility and starch hydrolysis index of *ca.* 70% and 77%, respectively. This study demonstrates the potential of fermentation to convert maize bran and germ, commonly-considered food wastes, into nutritive improvers, meeting nutritional and sensory requests of modern consumers.

O136 Tailored biotransformation of apple by-products as a source of nutritional supplement

Ali Zein Alabiden Tlais[1], Raffaella di Cagno[1], Pasquale Filannino[2], Olimpia Vincentini[3], Sascha Rohn[4], Andrea Polo[1], Marco Gobbetti[1]

[1]Free University of Bolzano, Food Engineering and Biotechnology, Bolzano, Italy
[2]University of Bari, Soil, plant and food department, Italy
[3]Istituto Superiore della Sanita (ISS), Department of food safety and veterinary puplic health, Italy
[4]University of Hamburg, Institute of food and environmental research potsdam, Germany

South Tyrol is the largest European producers of apples, where fruit processing generates a consistent volume of apple by-products that needs to be disposed. The huge volume of the low-cost apple by-products offers economical perspectives due to the presence of potentially biogenic valuable components. Chemical industries are now involving microbial fermentation to mediate the chemical conversion to produce industrially important compounds.

This study aims to develop tailored bioprocessing technologies, which include the combination of selected microbes to metabolize cholorogenic acid, one of the most abundant phenolics of apples. It can be trasformed to quinic acid and subsequently to shikimic acid, an industrially important compound that is used as key ingredient for the formulation of the drug Oseltamivir phosphate, emplied to treat swine/avian flu.

Chemical and microbiological composition of apple by-products, as well as the selection of appropriate pre-treatments, and the most promising microbes were investigated. According to the main target, the most suitable processing conditions was set-up. The analyses of the profiles of phenolics and organic acids establish the main modifications occurring during bioprocessing. Extraction and purification of biogenic compounds was performed through a multi-technology approach, and their exploitation was carried out through in vitro assay on human line cells.

Pre-treatments of apple by-products might be suitable to optimize the microbial fermentation. Microbes suitable to allow the quinic acid reduction pathway and favor the production of shikimic acid were selected. Sustainable shikimate extraction and purification procedures might favor economic and green process, and be a source of potential human functionality.

O137 Date-seeds flour as value added ingredient for sourdough bread

pasquale filannino[1], raffaella di cagno[2], Vincenzo Cantatore[1], hana ameur[2], ivana cavoski[3], marco gobbetti[2]

[1]*Università degli Studi di Bari, Soil Sciences, of Plants and Food, Bari, Italy*
[2]*Free University of Bozen-Bolzano, Science and Technology Faculty, Brixen, Italy*
[3]*IAMB, Valenzano, Italy*

Background: Date (*Phoenix dactylifera* L.) seeds are by-products of dates–processing industries and represent nearly 10–15% of the total weight of the fruit. Thus, date–seeds represent a cheap and easily recoverable source of bioactive compounds and dietary fiber. Date–seeds flour has a potential to be used in bread–making industries to develop novel functional food with higher amounts of fiber and others bioactive compounds with respect to the regular bread, fulfilling the consumer's demand for healthy food.

Objectives: The aim of this study was to setting up the protocol based on sourdough biotechnology to exploit date seeds flour as ingredient for sourdough bread.

Methods: Autochthonous strains of lactic acid bacteria and yeasts were selected for pro-technological and functional features, and used as starters to get date–seeds flour sourdough. Bread based on date–seeds flour sourdough was compared to conventional bread to highlight the strengthening points of the novel ingredient.

Results: The combined use of date–seeds flour and sourdough biotechnology improved nutritional, textural and sensorial features of bread.

O138 Biotransformation of waste bread using sourdough technology

Mikko Immonen[1], Yaqin Wang[2], Hanna Nihtilä[2], Rossana Coda[2], Ndegwa Henry Maina[2], Kati Katina[2]

[1]*University of Helsinki, Department of Food and Nutrition, Helsinki, Finland*
[1]*University of Helsinki, Department of Food and Nutrition, Helsinki, Finland*

Background: Significant amounts of bread waste is formed in the bakery industry due to several reasons such as excess production and system failures. Currently, waste bread is mainly used for non-food purposes, like animal feed and bioethanol feedstock. To match the modern demands for sustainable food chain and circular economy, food waste formation should be minimized and food-grade materials re-utilized for human nutrition.

Objectives: The aim of this study was to use enzymatic and microbiological bioprocessing to transform waste bread into a functional baking ingredient containing exopolysaccharides (EPS).

Methods: Carbohydrase enzymes and EPS-producing lactic acid bacteria (i.e. β-glucan and dextran) were used to hydrolyze and/or ferment the waste bread. Processed waste bread was used as an ingredient for new bread doughs. The breads were evaluated by measuring bread quality parameters; volume, hardness and staling rate.

Results: Waste bread direct mechanical slurrification and addition to new bread dough yielded significantly impaired products in terms of bread quality parameters. Enzymatically hydrolysed waste bread addition to new bread dough did not significantly modify bread quality, however, the high sugar content limited the recycling amount. Fermentation of waste bread with dextran-producing LAB *Weissella confusa* helped to maintain bread quality even when higher waste bread addition was used. In turn, fermentation with β-glucan producing *Pediococcus claussenii* did not contribute to bread quality. Based on our results, waste bread can be recycled within the bakery when suitable bioprocessing, starch hydrolysis and/or dextran-forming fermentation, is being applied.

O139 Acute RNA virus infections: Are they cleared?

Diane Griffin[1]

[1]*Johns Hopkins Bloomberg School of Public Health, Molecular Microbiology and Immunology, Baltimore Maryland, United States*

Background: Many RNA viruses cause acute infections with recovery within days or weeks after which immunity is established and infectious virus can no longer be recovered. However, our studies with alphavirus encephalomyelitis and measles have shown that viral RNA remains detectable for prolonged periods of time.

Objectives: To determine the state, location and consequnces of persistent viral RNA after acute infection.

Methods: Sindbis virus RNA in the brains of infected mice was localized with fluorescence in situ hybridization and the viral sequences characterized. Measles virus RNA in peripheral blood of infected rhesus macaques was identified and characterized by virus-inclusive single cell sequencing. Immune responses were measured by ELISPOT assays for T cells and EIA and neutralization for antibody.

Results: For alphavirus infections of the brain and spinal cord RNA persistence is associated with continued local production of antibody and residence of T cells. For measles, RNA persists in lymphoid and myeloid cells in blood and lymphatic tissue and is associated with ongoing stimulation and maturation of the immune response that leads to life-long protective immunity. RNA persistence also leads to a risk for virus reactivation, renewed transmission and development of late disease (*e.g.* subacute sclerosing panencephalitis years after apparent recovery from acute measles virus infection).

O140 Staphylococcal survival in the host

Andrew Edwards[1]

[1]*Imperial College London, MRC Centre for Molecular Bacteriology and Infection, London, United Kingdom*

Background: *Staphylococcus aureus* is responsible for a raft of infections, including bacteraemia, infective endocarditis and osteomyelitis. These infections are often hard to treat and frequently become chronic or recurrent. The persistence of *S. aureus* in host tissues is typically associated with genotypic and phenotypic changes that reduce bacterial virulence but enhance resistance to host defences and/or antibiotics.

Objectives: To understand the host and bacterial factors that drive genetic diversity and how the resulting phenotypes contribute to staphylococcal survival in the host.

Methods: We have employed a range of assays, including *ex vivo* whole human blood models, gene expression reporter strains, antibiotic survival and mutation rate analysis.

Results: Our data indicate that a range of infection-relevant stresses cause bacterial DNA damage, including the oxidative burst of neutrophils and antibiotics. Repair of this damage is essential for bacterial survival and leads to an increased mutation rate via the SOS response. The resulting emergence of small colony variants, via mutations within genes that encode components of the electron transport chain, promotes staphylococcal survival by conferring resistance to neutrophil-mediated killing. Another common phenotype, the loss of quorum-sensing activity, resulted in bacerial survival during exposure to daptomycin. In summary, staphylococcal DNA repair processes are central to both pathogen survival and host adaptaton by promoting resistance to both host defences and antibiotics. Pharmacological inhibition of DNA repair could, therefore, provide a useful therapeutic approach.

O141 Transcriptomics reveal the immune response in tuberculosis: *Type I IFN response in tuberculosis: foe & occasionally friend!*

Anne O'Garra[1]

[1]*The Francis Crick Institute, United Kingdom*

Background: Tuberculosis (TB) caused by infection with Mycobacterium tuberculosis (M. tuberculosis), is a major cause of morbidity and mortality worldwide. Efforts to control tuberculosis are hampered by difficulties with diagnosis, prevention and treatment. Most people infected with M. tuberculosis remain asymptomatic/latent with a 10% lifetime risk of developing active tuberculosis disease.

Objectives: To determine whether a blood transcriptional signature can distinguish individuals with active TB from latent individuals who have been exposed/infected but remain asymptomatic.

Methods: Blood was obtained from patients with active TB, healthy controls and latent individuals and processed for RNA expression by microarray or RNA-Seq, and in-depth bioinformatic analyses.

Results: We identified a whole blood transcript signature for active tuberculosis correlating with the radiological extent of disease which diminished upon effective treatment. Blood transcriptomic analyses revealed that the tuberculosis signature was dominated by a neutrophil-driven interferon (IFN)-inducible gene profile, and suggested a hitherto under-appreciated role of Type I IFNαβ signalling in human tuberculosis pathogenesis. Our subsequent studies now provide further knowledge regarding the heterogeneity of this signature in some but not all asymptomatic tuberculosis contacts and how this relates to their protection or development of disease. We have also data on potential mechanisms underlying the contribution of type I IFN to the development of tuberculosis and diseases caused by other bacterial infections and the impairment of protective responses and these will be discussed in depth.

AOG is funded by The Francis Crick Institute, UK, ERC-2011-AdG, 294682-TB-PATH, BioAster Technology Institute. AOG thanks all who have contributed to this work.

O142 Mechanisms of host colonization by the commensal and pathogenic yeast Candida albicans

J. Christian Perez[1]

[1]*University Hospital Wuerzburg, Interdisciplinary Center for Clinical Research, Wuerzburg, Germany*

Background: The fungus *Candida albicans* is a leading cause of mucosal disease and life-threatening infections in immunocompromised individuals. Yet this yeast resides in the gastrointestinal tract of most, if not all, human adults, seemingly living there as a harmless commensal. *C. albicans* has no known environmental reservoir suggesting that it has extensively co-evolved with the mammalian host.

Objectives: To uncover *C. albicans* gene circuits governing the proliferation of the fungus in diverse host niches.

Methods: My laboratory has used mouse models of intestinal colonization and oral candidiasis to screen a library of transcription regulator deletion strains. Through RNA-Seq and ChIP-Seq experiments, we are establishing the targets of regulation of the "hits" and the biological processes that they govern.

Results: We have identified about a dozen transcription regulators that play roles in at least one of the murine models evaluated. In my presentation, I will describe in detail some of the gene circuits that we have discovered and how they contribute to the interplay with the host.

O143 Marine sediments microorganisms and the evolution of symbiotic lineages

Daniel Tamarit[1;2], Jennah E. Dharamshi[1], Laura Eme[1;3], Courtney W. Stairs[1], Thijs J. G. Ettema[1;2]

[1]*Uppsala University, Sweden*
[2]*Wageningen University & Research, Netherlands*
[3]*Paris-Sud University, France*

The establishment of symbiotic interactions has a remarkable evolutionary impact on microbial lineages. Insights into how symbioses form can come from the study of symbiotic features, as well as from the exploration of microbial diversity related to successful symbiotic groups. In this talk, I will briefly discuss ongoing research on genomic data from marine sediment microorganisms, and how it affects our understanding on the evolution of the Chlamydiae phylum and of the Eukaryotic domain. Finally, I will take the opportunity to introduce the Microbial Symbioses thematic series, and share my experience as handling editor.

O144 Population genomics of Lactobacillus kunkeei: a bee symbiont or a beehive resident

Andrea Garcia Montaner[1], Kristina Näslund[1], Matthew Webster[2], Siv G.E. Andersson[1]

[1]*Uppsala University, Cell and Molecular Biology, Uppsala, Sweden*
[2]*Uppsala University, Medical Biochemistry and Microbiology, Uppsala, Sweden*

The honeybee gut microbiome consists of eight bacterial species that contribute to different aspects of the host's physiology and metabolism. Lactobacillus kunkeei is the dominant species in the honey crop, also found in nectar, pollen and bee products. It is therefore thought to belong to the beehive microbiome. We analyzed 42 new L. kunkeei strains to understand the evolutionary processes that shape the genome, and intra-species genetic variation. Bacterial isolates were obtained from the honey crop of honeybees from two locations, with and without presence of the Varroa mite parasite. Phylogenomic reconstructions indicated higher strain diversity among samples from the mite-free area, although this correlation needs further investigation. Gene synteny is maintained across chromosomes, with gene content variation mainly affecting metabolic functions, possibly reflecting complementarity among strains co-habiting the same host. The analyses also provided indications of recombination across genomes, with great dynamism among the giant genes that are unique to his species and its closest relative, Fhon13. The giant genes are 9,3 to 25 kb in size, and with 4 to 5 genes in tandem they represent 10% of the chromosome size, and are potentially involved in adhesion to the host's epithelium. Finally, we explored the diversity of mobile genetic elements, which represent a great source of gene innovation and potential adaptations. Understanding genome evolution of L. kunkeei and the variability of its accessory genes are the first steps to unraveling its niche and potential role as a honeybee symbiont.

O145 Distinct Evolutionary Paths of Co-occurring Symbionts

Andreas Schramm[1], Marie Braad Lund[1]

[1]*Aarhus University, Department of Bioscience, Section for Microbiology, Denmark*

Background: Long-term symbiotic association between bacteria and eukaryotic hosts can result in extreme mutual dependency of the involved partners, including strong genome reduction or complete nutritional dependence. Symbiont function and transmission between hosts have been suggested to explain variation in host dependence across symbioses. The nephridia of many earthworm species harbor three specific, extracellular bacterial symbionts; *Verminephrobacter, Ca.* Nephrothrix, and *Ca.* Lumbricidophila. All three are vertically transmitted via deposition into the cocoon, where they colonize the developing embryo. *Verminephrobacter* benefits host reproduction and has co-evolved with earthworms for approx. 100 million years; the other symbionts are evolutionarily younger but still benefit host maturation.

Objectives: To assess the role of fine-scale differences in symbiont transmission, function, and microhabitat for the symbionts' evolutionary trajectory, using the nephridial symbiosis of earthworms as model.

Methods: Symbiont genomes (from pure cultures for *Verminephrobacter* and cocoon metagenomes for the other two symbionts) were reconstructed and analyzed for genome reduction, AT-bias, and dN/dS ratio and codon usage bias as indicators of the level of purifying selection and genetic drift.

Results: Surprisingly, the youngest symbiont (*Ca.* Lumbricidophila) had the most reduced genome, with stronger signs of bottleneck-induced drift than the evolutionary oldest symbiont, *Verminephrobacter; Ca.* Nephrothrix showed high microdiversity and no indications of genome degradation. We could trace these differences to the fidelity and stringency of the symbionts' vertical transmission and to the main site of symbiont proliferation. Our data emphasize how subtle differences in transmission and function drive genome evolution of symbionts, even in a single host.

O146 Christensenella and Blautia differentially impact host metabolism in a gnotobiotic mouse model

Jillian Waters[1], James Marsh[1], Nicholas Youngblut[1], Ruth Ley[1]

[1]*Max-Planck-Institute for Developmental Biology, Department of Microbiome Science, Tuebingen, Germany*

Christensenella minuta is a member of the family Christensenellaceae, which our group recently established as highly heritable (i.e., influenced by host genetics) and is associated with a lean BMI in humans. We also performed transplantation studies using germfree mice, demonstrating that addition of live *C. minuta* to obese human donor stool significantly reduces weight and adiposity gains compared to mice receiving the same unamended donor stool. The mode of action by which *C. minuta* regulates host metabolism remains to be elucidated.

Here, we address the question of how *C. minuta* impacts host phenotype using a simplified gnotobiotic mouse model. We designed experiments in which gnotobiotic mice were mono-associated with *C. minuta,* with *Blautia hydrogenotrophica,* or with both. We have shown *C. minuta* produces copious hydrogen, which can be used as a substrate by *B. hydrogenotrophica* to produce acetate. As such, we hypothesized that more acetate would be detected in the ceca of dual-associated mice*,* and would result in increased adiposity. Consistent with our hypothesis, we observed that dual-associated mice had a significant increase in body fat percentage. Further, short chain fatty acid (SCFA) analysis of cecal contents also supports this hypothesis, as acetate and other SCFAs were elevated in dual-associated mice. Untargeted metabolomics on cecal contents revealed some notable metabolites that differentiated treatment groups. RNA-sequencing of various host tissues reveals global changes regarding carbon metabolism and oxidative phosphorylation based on colonization of *C. minuta*. These results suggest that *C. minuta* interactions with other microbes strongly regulate host phenotypes.

O147 Less is More: Myth and Reality of Chronic Infection with Borrelia Burgdorferi

Eugene Shapiro[1]

[1]*Yale University, Pediatrics; Epidemiology of Microbial Diseases, New Haven, United States*

Background: Lyme disease is caused by *Borrelia burgdorferi* sensu latu. While there is evidence that chronic infection (eg, *acrodermatitis chronica atrophicans*) occurs occassionally in humans who are not appropriately treated with an antimicrobial, there are vocal advocate groups, which include patients and "Lyme-literate" health care providers, that claim that chronic infection with *B. burgdorferi* is common and is both difficult to diagnose and difficult to treat with conventional courses of antimicrobials.

Objectives: To present the evidence about chronic infecton with *B. burgdorferi.*

Methods: Evidence for the existence of chronic infecton with *B. burgdorferi* was reviewed.

Results: There are some unusual features of infection with *B. burgdorferi* in untreated patients, such as the relatively long delay between initial infection and the onset of Lyme arthritis as well as chronic infection as occurs in *acrodermatitis chronica atrophicans*. There is no evidence either that viable bacteria persist after conventional courses of antimicrobial treatment or that patients with chronic, non-specific symptoms are suffering from chronic infection with *B. burgdorferi*. There is considerable evidence that these patients do not have chronic infection with *B. burgdorferi*.

O148 Molecular mechanisms used by bacteria for host colonisation and biofilm formation

Jason Paxman[1], Alvin Lo[2], Santosh Panjikar[3], Matthew Sullivan[4], Mike Kuiper[5], Glen Ulett[4], Mark Schembri[2], Begona Heras[1]

[1]*La Trobe University, Biochemistry and Genetics, Melbourne, Australia*
[2]*The University of Queensland , School of Chemistry and Molecular Biosciences, Brisbane, Australia*
[3]*Australian Synchrotron, Macromolecular Crystallography, Melbourne, Australia*
[4]*Griffith University, School of Medical Science, and Menzies Health Institute , Gold Coast, Australia*
[5]*Csiro, Molecular & Materials Modelling group Data61, Melbourne, Australia*

Background: So little is known about how bacteria utilise surface adhesins to adhere and colonise host surfaces, despite this process being critical for bacterial infections, the contamination of food and medical devices along with the formation of persistent bacterial biofilms. The largest group of these adhesins are the non-fimbrial autotransporter adhesins, whereby until our research almost nothing was known about their structures and molecular mechanisms of action.

Objectives: We sought to uncover the structures, mode of action, regulation and roles in bacterial pathogenesis of autotransporter adhesins.

Methods: A multidisciplinary approach of X-ray crystallography with biophysical, biochemical and microbiology methods was used along with testing in animal models.

Results: We now show that these proteins form long >500 residue β-helix structures that incorporate different features to allow binding to their targets. So far we have found these adhesins fall into 2 mechanistic groups (i) those such as Antigen 43 from widespread pathogenic *E. coli* strains that promote bacterial aggregation/biofilm formation by self-interactions between neighbouring bacteria and (ii) others such as UpaB from Uropathogenic *E. coli* that through significant structural modifications are able directly bind host surfaces. Remarkably, we have now found that other adhesins such as Entertoxigenic *E. coli* TibA, can switch between these functions by novel post-translational modifications.

This research is uncovering new structures and modes of action for the autotransporter adhesins to finally reveal how bacteria intimately bind to surfaces. Importantly, we are using our findings to develop new classes of anti-bacterial inhibitors including our recently patented biofilm inhibitor.

O149 Autotransporter Adhesins in the fish pathogen Yersinia ruckeri

Agnieszka Wrobel[1], Jack Leo[1], Stephan Göttig[2], Dirk Linke[3]

[1]*Department of Biosciences, Oslo, Norway*
[2]*University Hospital, University of Frankfurt, Frankfurt, Germany*
[3]*Department of Biosciences, University of Oslo, Oslo, Norway*

Background: *Yersinia ruckeri* is a fish pathogen of high exonomic importance. It infects mainly salmon and trout, and causes huge losses in the aquaculture industry. One of the problems with *Yersinia ruckeri* infections in closed aquaculture systems is its ability to form biofilms in pipes and filters that are difficult to sanitize.

Objectives: Our aim was to identify bacterial adhesin genes in the genome that could be responsible for biofilm formation

Methods: We used genome sequencing on a Norwegian outbreak strain of *Yersinia ruckeri*. Using bioinformatics tools, we tried to identify adhesins that would be similar to well-studied adhesins in *Yersinia enterocolitica*, a human gut pathogen for which much more data on virulence factors exists.

Results: We found several inverse autotransporter adhesins in *Y. ruckeri* genomes in our own sequencing data. When studying them in detail, we found that in many cases in public databeses, these genes were annotated with wrong repeat numbers. This is based on the underlying seqeuncing technologies: only PacBio sequencing was able to read long stretches of identical repeats correctly. In addition, we annotated a plasmid that contains a type IV conjugative pilus, and that might be directly involved in virulence. Using qPCR, we established the growth conditions unde which the adhesin genes are expressed. Using advanced microscopy techniques, we showed that both autotransporters in *Y. ruckeri* contribute to biofilm formation. In a Galleria infection model, single-gene knockouts were less virulent than the wildtype for both adhesins, suggesting a direct role in the infection process.

O150 New insights into Pseudomonas aeruginosa adaptive response to sublethal concentrations of tobramycin

Ali Tahrioui[1], Rachel Duchesne[1], Emeline Bouffartigues[1], Sophie Rodrigues[1], Olivier Maillot[1], Damien Tortuel[1], Julie Hardouin[2], Laure Taupin[3], Marie-Christine Groleau[4], Alain Dufour[3], Eric Déziel[4], Marc Feuilloley[1], Nicole Orange[1], Olivier Lesouhaitier[1], Pierre Cornelis[1], Sylvie Chevalier[1]

[1], Laboratory of Microbiology, Signals and Microenvironment, LMSM EA4312, Évreux, France
[2], Laboratory of Polymers, Biopolymers, and Surfaces, UMR 6270 , Rouen, France
[3], Laboratory of Marine Biotechnology and Chemistry, LBCM, EA3884, Lorient, France
[4], Centre INRS-Institut Armand-Frappier, Laval (Québec), Canada

Background: Biofilms are structured microbial communities that are the leading cause of numerous chronic infections which are difficult to eradicate. Within the lungs of individuals with cystic fibrosis, *Pseudomonas aeruginosa* causes persistent biofilm infection that is commonly treated with aminoglycoside antibiotics such as tobramycin. However, exposure to sublethal concentrations of the aminoglycoside tobramycin were previously shown to increase biofilm formation by *P. aeruginosa*

Objectives: In the current study, we sought to elucidate potential adaptive mechanisms shaping the tobramycin-enhanced biofilm formation in *P. aeruginosa*.

Methods: We combined confocal laser scanning microscope analyses, proteomics profiling, gene expression assays and phenotypic studies to unravel *P. aeruginosa* potential adaptive mechanisms in response to tobramycin exposure during biofilm growth.

Results: We show that the modified biofilm architecture is related at least in part to increased extracellular DNA (eDNA) release, most likely as a result of biofilm cell death. Furthermore, the activity of quorum sensing (QS) systems was increased, leading to higher production of QS molecules. We also demonstrate upon tobramycin exposure an increase in expression of the small RNAs PrrF1 and PrrF2, known for promoting 4-hydroxy-2-alkylquinolines production, as well as expression of iron uptake systems. Remarkably, biofilm biovolumes and eDNA relative abundances in *pqs* and *prrF1,F2* mutant strains decrease in the presence of tobramycin. Overall, our findings offer experimental evidences for a potential adaptive mechanism linking PrrF1/F2 sRNAs, QS signaling, biofilm cell death, eDNA release, and tobramycin-enhanced biofilm formation in *P. aeruginosa*.

O151 Latent and symptomatic Pectobacterium atrosepticum-caused plant infections: ultrastructure, biochemistry and transcriptomics

Vladimir Gorshkov[1;2], Rim Gubaev[1], Olga Petrova[1], Marina Ageeva[1], Natalia Gogoleva[1;2], Olga Parfirova[1;2], Bakhtiyar Islamov[1], Vladimir Vorob'ev[1;2], Polina Mikshina[1], Yuri Gogolev[1;2]

[1]Kazan Institute of Biochemistry and Biophysics, FRC Kazan Scientific Center, Russian Academy of Sciences, Kazan, Russian Federation
[2]Kazan Federal University, Kazan, Russian Federation

Background: The harmful phytopathogen *Pectobacterium atrosepticum* (*Pba*) is known to utilize not only brute force for host-plant colonization causing soft rots, but also a stealth behavior leading to asymptomatic infections.

Objectives: The aim of our study is to differentiate physiological and molecular criteria of stealth and brute force behavior of *Pba*, which may provide a significant basis for the control of soft rots.

Methods: Different types of microscopy and NGS-transcriptomics constitute the basis of our systemic methodology providing information on 1) spatial organization of microbial population in planta and phenotypic diversity of *Pba* cells – both are the criteria of the infection type; and 2) plant and bacterial genes differentially expressed during latent and acute infection enabling us to assume the molecular players (including novel ones) that determine the type and outcome of plant-pathogen interaction. Then, specific test-systems (including those involving mutagenesis, cloning, chromatography, NMR-spectroscopy, phytohormone application, immunodetection, etc.) are developed to experimentally characterize these players.

Results: The obtained results have led to the notion that latent infections (without significant plant-host fitness cost) are likely to be norm and reflect natural equilibrium between plants and pathogens, and the development of pathological processes is a result of the disturbance of this equilibrium due to the specific physiological reactions of both organisms. Special role in determination of the infection type is played by phytohormonal status, which is actively modulated by the pathogen as well as *Pba*-induced plant-mediated reorganization of the plant cell wall. The study is supported by RSF (No 19-14-00194).

O152 'Specialised' translation underlies adaptation of the human pathogen Mycobacterium tuberculosis

Anna Grabowska[1], Elizabeth Sawyer[1], Teresa Cortes[1]

[1]*LSHTM, London, United Kingdom*

Background: It is estimated that more than ¼ of human population is infected with *Mycobacterium tuberculosis* (MTB). Only 5-10% of infected individuals develop active disease, whereas in 90-95% of the cases the infection remains in asymptomatic 'latent' state even over decades and constitutes reservoir for re-activation and spread of the disease. Latency is linked to a complex gene expression regulation in MTB, even though particular mechanisms underlying metabolic switching between replicating and non-replicating state, detected in active and latent disease, are so far poorly understood. Evidence from experiments on antibiotic-induced stress in *Escherichia coli* suggest that metabolic re-programming could occur at the translational level.

Objectives: Given significantly higher proportion of leaderless transcripts in MTB than in other pathogenic bacteria, we aim to characterize the translation pattern of leaderless versus canonical Shine-Dalgarno transcripts, and find factors responsible for differential translation of these two types of mRNA.

Methods: We constructed a set of MTB reporter strains carrying Shine-Dalgarno or leaderless versions of firefly luciferase gene and used them in various experiments, reflecting optimal and non-optimal (infection-associated) conditions of growth.

Results: We observed efficient translation of MTB leaderless mRNAs in all tested conditions of growth. In some stress conditions, i.e. in starvation model of growth arrest, MTB leaderless translation was preferentially enhanced, compared to SD transcripts. As leaderless transcripts in MTB encode proteins with secondary adaptive functions, like toxin-antitoxin systems, known to be activated in ‘persistence’ models, translation ‘specialization’ could be the mechanism underlying switching to latency and ‘persistence’ in MTB.

O153 The response regulator-encoding gene uvrY of Escherichia coli is under positive selection upon prolonged growth in the urinary tract

Elisabed Zaldastanishvili[1], Birgit Ewert[1], Roswitha Schiller[1], Claudia Acquisti[2], Ulrich Dobrindt[1]

[1]*Institute of Hygiene, Münster, Germany*
[2]*Institute of Evolution and Biodiversity, Münster, Germany*

Background: Urinary tract infection (UTI) is the most frequently occurring bacterial infection in industrialized countries. Uropathogenic *E. coli* (UPEC) can cause symptomatic UTI, but so-called asymptomatic bacteriuria (ABU) *E. coli* can also colonize the urinary tract without provoking an overt host response. The determinants coding for the BarA-UvrY two-component system (TCS) exhibit nucleotide sequence variability upon prolonged growth in the bladder.

Objectives: The global role of the BarA/UvrY TCS in metabolic adaptation and control of different fitness-related functions led us to hypothesize that positive selection shapes this TCS resulting in increased fitness in the urinary tract. We propose convergent evolution of the BarA/UvrY TCS in natural *E. coli* populations grown in urine towards an improved fitness in urine.

Methods: To prove that *barA* or *uvrY* are under positive selection in UPEC, we analyzed their nucleotide sequence variability in *E. coli* isolates from humans with ABU, symptomatic UTI and from feces of healthy individuals. The McDonald-Kreitman test was used to detect signatures of natural selection. Competitiveness of *E. coli* MG1655 carrying different *uvrY* alleles was compared in gluconeogenic media such as LB and urine. The fitness effect was analyzed *in vitro* and *in vivo*.

Results: We demonstrate that *uvrY* is under positive selection upon long-term growth in the urinary tract. Competition experiments with isogenic strains carrying different *uvrY* alleles revealed that abolished or altered UvrY functionality can lead to a fitness advantage. We ruled out the contribution of gluconeogenic pathways to the observed phenotypic differences.

O154 What did algal viruses do for us anyway (apart from establishing an evolutionary path to eukaryotic life)?

Willie Wilson[1]

[1]*Marine Biological Association, The Laboratory, Plymouth, United Kingdom*

Background: Every day, every moment, an epic battle is raging across the globe. It's happening in the ocean. The evidence is both highly visible and totally hidden, depending on your perspective.

Objectives: In this talk, Willie will discuss the tale of an arms race involving trillions of sea creatures and why their struggle is vital to life on this planet.

Methods: A group of phytoplankton, known as coccolithophores, are engaged in a surprisingly complicated arms race with deadly giant viruses. A virus is problematic enough when you're a human. Now imagine being a single-cell alga and mixing it up with the hugest virus you've ever seen. The coccolithophores are outgunned, but they won't go down without a serious fight. The talk will start with a RadioLab interview (previously aired on National Public Radio in the US) by reporter Ari Daniel Shapiro who visited Willie to explain how our itsy-bitsy heroes take arms against a sea of troubles.

Results: Their discussion explores how this battle, and others like it, makes life on Earth possible. Willie will continue the dialogue using examples of his research on the coccolithoviruses and development of single-virus genomics approaches to study them and other virus leviathans.

O155 Distinct cyanophage distribution and infection patterns across environmental gradients in the oceans

Debbie Lindell[1], Michael Carlson[1], Yotam Hulata[1], Nava Baran[1], Svetlana Goldin[1], Nitzan Shamir[1], Ilia Maidanik[1], Noor Marwat[1], Francois Ribalet[2], Oscar Sosa[3], Sara Ferron[3], Angelique White[3], David Karl[3], E. Virginia Armbrust[2]

[1]*Technion - Israel Inst. Technology, Biology, Haifa, Israel*
[2]*University of Washington, School of Oceanography*
[3]*University of Hawaii, School of Ocean and Earth Science*

Background: Viruses are globally abundant and highly diverse in their genetic make-up and in the hosts they infect. They influence the abundance, diversity and evolution of their hosts as well as the biogeochemical cycling of matter in the oceans.

Objectives: However, lack of quantitative measurements of viruses and the extent of viral infection at taxonomically meaningful levels have precluded a quantitative understanding of their impact on these processes.

Methods: Towards this end, we have developed the polony method, a single molecule solid-phase PCR method, to measure abundances of three cyanophage families and the extent to which they infect the unicellular marine cyanobacteria, *Synechococcus* and *Prochlorococcus.*

Results: Here I will report recent findings showing distinct patterns in cyanophage abundances, community composition and infection along surface transects from subtropical to subpolar waters in the North Pacific Ocean. Our findings highlight how changing environmental conditions found in distinct oceanic regimes affect the ecology of cyanophages and their cyanobacterial hosts.

O156 The Baltic Sea virome: Cultures, communities, and consequences

Karin Holmfeldt[1]

[1]*Linnaeus University, Centre for Ecology and Evolution in Microbial Model Systems, Kalmar, Sweden*

Background: Through host-specific infection and lysis, viruses that infect bacteria (phages) impact their host's diversity and mortality, with effects on global biogeochemical cycles. Further, aquatic phages have been shown to have a direct impact on biogeochemical cycles by forcing the host to express phage derived metabolic genes.

Objectives: However, there is still a paucity in our knowledge regarding taxonomy, host interactions, and functional potential of viruses in aquatic environments, the Baltic Sea included.

Methods: Through a combination of 'omics and experimentation of isolated phage-bacteria model systems and high-throughput sequencing of viral communities from the Baltic Sea, in-depth information regarding phage diversity, functionality, interactions, and temporal dynamics could be gained.

Results: While phages infecting some bacterial species showed a large taxonomic variety with different viral genera and even families, other bacterial species could only be infected by highly similar phages. Comparison to metagenomic time series showed yearly temporal trends among the phages, which corelated with their potential hosts. The phages varied in host specificity from highly host-specific, only infecting the strain they were isolated on, to phages infecting a large number of different strains. However, the efficiency of infection varied with several orders of magnitude and had the potential to change depending on which host was latest infected. This created a mosaic phage-host complexity which have ecological implications and is difficult to elucidate using only community 'omics data. Overall, the combination of culture-experiments and 'omics studies of natural communities provides a unique platform for novel discoveries and hypothesis testing.

O157 Unravelling the vast diversity of vibrioophages by genomic and metagenomic analyses

Slawomir Michniewski[1], David Scanlan[2], Andrew Millard[3]

[1]*University of Warwick, Warwick Medical School, United Kingdom*
[2]*University of Warwick, School of Life Sciences, United Kingdom*
[3]*University of Leicester, Department Genetics and Genome Biology, United Kingdom*

Background: The *Vibrio* genus is genetically and metabolically diverse comprising a major component of the marine microbial community and with many species associated with diseases of humans, fish and shellfish. Bacteriophages that infect *Vibrio* spp. have been shown to be genetically diverse, including the recent discovery of a new family of non-tailed phages (*Autolykiviridae*). Despite this recent finding, the diversity of *Vibrio* prophages, and the genes that they carry, remains largely unknown.

Objectives: Assess the diversity of prophages within the genus *Vibrio*.
Identify auxiliary metabolic genes within vibriophages.

Methods: The genomes of ~7000 Vibrios were assembled and prophage regions were predicted. Prophage regions were compared in an all-v-all approach and clustered based on mash distance. All prophages were annotated with Prokka using a database of curated phage proteins. Database searching was used to identify auxiliary metabolic genes. Selected genes were expressed in *E. coli* to confirm their predicted function. By mapping reads from viral metagenomes to representative prophages their distribution in the environment was assessed.

Results: We identified 1100 different prophage clusters across the *Vibrio* genus, with the majority of these containing prophages that cannot be ascribed to a current phage species. The majority of clusters were restricted to specific *Vibrio* spp. We identified an assortment of auxiliary metabolic genes within prophages, including predicted antibiotic resistance genes. We confirmed the function of antibiotic resistance genes by expression in *E. coli*.

O158 Widespread distribution of class II membrane fusion proteins in viruses and cells

Felix Rey[1]

[1]*Institut Pasteur, Virology, Paris, France*

Structural studies have revealed that the membrane fusion proteins used by enveloped viruses to enter cells belong to one of three structural classes (I, II and III) of homologous proteins, irrespective of the virus taxonomy based on the polymerase gene. This finding illustrates the mosaic nature of viral genomes, which are a collection of genes derived from different origins. One special case is that of the class II fusion proteins found in the *Togaviridae*, *Flaviviridae* and in several families of the newly defined *Bunyavirales* order. Class II fusion proteins were also detected in *C. elegans* retroviruses, which are otherwise clearly related to mammalian retroviruses through the Gag-Pol gene, although mammalian retrovirus have an envelope protein belonging to class I. The cell-cell fusion protein EFF-1, responsible for syncytia formation to form the skin during *C. elegans* embryogenesis, is also homologous to the class II viral proteins. More recently, the ancestral gamete fusogen HAP2 was identified as belonging to class II, and is further illustration of the impact of virus-cell genetic exchanges. This interchange appears to have been at the origin of sexual life on earth by inducing the specific merger of the plasma membranes of sperm and egg during fertilization by the same mechanism used by enveloped viruses to infect cells. In this presentation, I will review the specific features of class II fusion proteins and their mechanism of action.

O159 Structural and biochemical characterisation of toxin-antitoxin system promoter binding by type IV antitoxins of Mycobacterium tuberculosis

Izaak Beck[1], Ben Usher[1], Tim Blower[1]

[1]*Durham University, Biosciences, Durham, United Kingdom*

Background: Tuberculosis remains a global health issue with an estimated 1.5 million deaths per year. Its causative agent, *Mycobacterium tuberculosis*, encodes an extraordinary amount of toxin-antitoxin systems (79 identified to date) of which, many remain uncharacterised. There are three putative type IV systems present in *M. tuberculosis* with the antitoxins denoted Rv2827c, Rv1044 and Rv0837c and their respective toxins appear to be significantly upregulated during infection. Rv2827c has been structurally classified and the homologous protein, AbiEi (*Streptococcus agalactiae*), has been functionally classified as an autoregulatory type IV antitoxin.

Objectives: To better understand the autoregulatory mechanisms of type IV toxin-antitoxin systems of *M. tuberculosis* by investigating their promoter binding capacities and respective kinetics. This will be complemented by the structural classification of the antitoxins.

Methods: Protein-promoter interaction studies have been performed using EMSAs. Structural work has been completed using X-ray crystallography and experimental phasing using selenomethionine labelled AbiEi protein crystals.

Results: The crystal structure of the autoregulatory antitoxin protein AbiEi has been solved to 1.83 Å and contains the wHTH DNA-binding domain as seen in Rv2827c. Rv2827c binds to four sites within the *rv2827c* promoter in a sequence dependent pseudo-negatively cooperative manner; a mechanism not seen before in toxin-antitoxin system autoregulation. In contrast, Rv1044 appears to not bind the cognate promoter region. Rv1044 can bind the *Streptococcal* AbiEi promoter, however, this appears to be artefactual as there is no cross-regulation between the mycobacterial type IV promoters.

O160 Architectural principles for Hfq/Crc-mediated regulation of genes during carbon catabolite repression in Pseudomonas aeruginosa

Udo Bläsi[1], Xue Yuan Pei[2], Tom Dendooven[2], Elisabeth Sonnleitner[1], Shaoxia Chen[3], Ben Luisi[2]

[1]*Max F. Perutz Laboratories, Microbiology and Genetics, Vienna, Austria*
[2]*University of Camebridge, Biochemistry, Camebridge, United Kingdom*
[3]*MRC Laboratory of Molecular Biology, Camebridge, United Kingdom*

Background: In *Pseudomonas aeruginosa* the RNA chaperone Hfq and the *c*atabolite *r*epression *c*ontrol protein (Crc) act as post-transcriptional regulators during carbon catabolite repression. Crc was shown to be required for full-fledged Hfq-mediated translational repression of catabolic genes. Biochemical and biophysical approaches indicated that Crc and Hfq form an assembly in the presence of RNAs containing A-rich motifs, and that Crc interacts with both, Hfq and RNA.

Objectives: To disclose how Crc augments translational repression by Hfq, structural studies were performed to reveal the architecture of the Hfq/Crc/RNA complex(es).

Methods: Cryo-EM was used for the structural analysis of Hfq/Crc/RNA complexes. Crc variants were constructed by genetic menas to validate the different Hfq/Crc/RNA complexes.

Results: The structure of the Hfq/Crc/RNA complex disclosed for the first time how an interacting protein can augment translational repression by Hfq. The core of the assembly is formed through interactions of two cognate RNAs, two Hfq hexamers and a Crc pair. Additional Crc protomers are recruited to form higher-order assemblies with demonstrated *in vivo* activity. A pattern of repeating motifs in the RNA supports the quaternary structure. This study reveals how Hfq cooperates with a partner protein to regulate translation and provides a structural basis for an RNA code to guide global regulators to interact cooperatively and regulate different RNA targets.

O161 A stretch/recoil allosteric mechanism controls the catalytic cycle of Rel enzymes

Abel Garcia-Pino[1]

[1]*Université Libre de Bruxelles, DBM, Gosselies, Belgium*

Background: Dormancy and persistence are intricately related with the stress response. (p)ppGpp is a key regulator of bacterial growth and stress tolerance that is synthetized and hydrolyzed by RelA/SpoT Homologue (RSH) enzymes. It has been proposed that nucleotides could play an important role in the regulation of (p)ppGpp levels by these enzymes.

Objectives: Characterization and analysis of the conformational cycle of the synthetase and hydrolase domains of the long RSH enzyme from *T. thermophylus* and its regulation by nucleotides during catalysis.

Methods: We provide structural and biophysical evidence at a single molecule level that nucleotide binding acts as an efficient switch that turns on/off ppGpp synthesis by coupling nucleotide binding to the RelA/SpoT conformational cycle.

Results: We solved the structures of ttRel in the active hydrolase and synthetase states and studied the conformational landscape of the enzyme by single molecule FRET microscopy. We show that in contrast with small alarmone synthetases (SAS) the binding of GDP precedes the incorporation of ATP and stabilizes an open state incompatible with ppGpp hydrolysis. By contrast the presence of ppGpp reverts the protein to a very compact state incompatible with ppGpp synthesis. These results highlight that nucleotides exert a stronger allosteric control on RSH enzymes than what was initially assumed by arranging the active site residues. Our structures could have important implications for the development of new antibacterials and underscore the complexity of the regulation of the catalytic cycle of these enzymes.

O162 Hijacking the hijackers: the satellite-helper pathogenicity island interaction

Andreas Haag[1], Magdalena Podkowik[1,2], Richard Novick[2], Jose Penades[1]

[1]*University of Glasgow, Institute of Infection, Immunity, and Inflammation, Glasgow, United Kingdom*
[2]*Skirball Institute Of Bio Molecular Medicine, New York, United States*

Background: The development of novel multi-resistant and hypervirulent bacterial strains from formerly avirulent or only weakly virulent strains is dramatically fuelled by the acquisition of mobile genetic elements carrying virulence factors. Despite its relevance, the mechanisms underlying gene transfer among bacteria remain, in most cases, unidentified. Staphylococcal pathogenicity islands (SaPIs) are the prototypical members of a novel family of mobile genetic elements, the phage-inducible chromosomal islands (PICIs). They are important because they carry and disseminate clinically relevant virulence genes, including toxins and antibiotic resistance genes. It has long been assumed that SaPI transfer depends exclusively on a helper phage.

Objectives: In this project we establish a novel pathway by which bacteria exchange genetic information and decipher the molecular basis of this unprecedented strategy.

Results: We demonstrate the existence of a hitherto unrecognised attribute of the SaPIs that allows their promiscuous spread in nature. We describe that a subset of SaPIs has evolved a fascinating strategy that promotes their high transfer by pirating other SaPIs, highlighting these elements as one of nature's most intriguing subcellular parasites. These findings represent the discovery of two novel subcellular entities, the helper and the satellite pathogenicity islands, whose mechanism of interaction and relevance in the emergence and dissemination of novel bacterial virulent clones remains to be determined.

O163 Computational modeling, molecular dynamic simulation and in vitro studies suggested that natural epiesteriol is a potential inhibitor against Omp38, RecA, PyrE and PyrF targets of multi-drug resistant Acinetobacter baumanni

Sinosh Skariyachan[1]

[1]*Dayananda Sagar College of Engineering, Biotechnology, Bangalore, India*

Background: Multidrug resistant *Acinetobacter baumannii* (MDRAb) declared as priority-I pathogen by WHO (2017) and screening of potential therapeutic agents has profound application.

Objectives: This study aimed to identify putative drug targets of MDRAb and validate the therapeutic potential of natural molecules by structure based drug screening and *in vitro* studies

Methods: Ten clinical isolates of Ab were subjected to antibiotic susceptibility testing against five carbapenems and two colistins. Based on the metabolic pathway and functional role analysis, outer membrane protein (*Omp38*), protein RecA (*RecA*), orotate phosphoribosyltransferase (*PyrE*) and orotidine 5'-phosphate decarboxylase (*PyrF*) were identified as potential drug targets by KEGG database search. The three dimensional structure of Omp38 was retrieved from PDB and others were computationally predicted and validated. 236 natural molecules were screened from various databases and subjected to virtual screening, molecular docking and molecular dynamic simulation. The therapeutic potential of computationally predicted molecules was validated by *in vitro* studies.

Results: The clinical isolates (n=10) showed extreme dug resistance to carbapenems and colistins ($p<0.05$). Computational screening suggested that 06 leads were qualified for drug likeliness, pharmacokinetic features and one molecule-natural epiesteriol (16b-Hydroxy-17a-estradiol) exhibited significant binding towards four drug targets in comparison with the binding of faropenem and polymyxin E towards their usual targets. MD simulations suggested that epiesteriol-receptor complexes demonstrated stability throughout the simulation. The growth curve and time kill assays revealed that MDRAb showed resistance to Faropenem and Polymyxin-E and purified epiesteriol showed significant inhibitory properties (100 µg/mL) towards four drug targets in comparison with the controls ($p\leq0.05$)

O164 The development of meningococcal vaccines

Ian Feavers[1]

[1]*National institute for Biological Standards and Control, Bacteriology, Potters Bar, United Kingdom*

Once feared for its rapid onset together with its severe morbidity and mortality, meningococcal disease is now largely controlled by effective vaccination programmes in most parts of the world. Even in the countries of sub-Saharan Africa, which in the past have been ravaged by repeated epidemics of group A meningococcal disease, today the problem is largely controlled by vaccination. Although the development of meningococcal vaccines has been challenging, it has benefitted from two important technical advances, namely polysaccharide conjugation and recombinant DNA technologies, as well as from a clearly defined serological correlate of protection. This presentation will review these developments and examine the potential to reduce the burden of disease further in the future.

O165 Vaccination against VZV; where are we now and what does the future hold?

Judith Breuer[1]

[1]*University College London, United Kingdom*

The diseases caused by Varicella zoster virus, chickenpox and shingles are now preventable by three good vaccines. However, many countries, including the UK, have not implemented VZV vaccination programmes. I discuss the reasons for this as well as some of the implications of preventing VZV disease for vaccination in the future.

O166 Progress and challenges in TB vaccines; a paediatric perspective

Elizabeth Whittaker[1;2]

[1]*Imperial College London, Paediatrics, London, United Kingdom*
[2]*Imperial College Healthcare NHS Trust, Paediatric Infectious Diseases, London, United Kingdom*

Children represent both a clinically important population susceptible to tuberculosis and a key group in whom to study intrinsic and vaccine-induced mechanisms of protection. The only licensed vaccine to prevent TB, BCG, is moderately effective in preventing TB in young children in particular from disseminated disease in high endemic settings, but is unreliable in preventing TB in adolescents and adults. Boosting the BCG vaccine response, either through BCG revaccination or with a novel vaccine, may have a beneficial role in high endemic settings. There has however been controversy surrounding the role of BCG revaccination aso initial data from a large adolescent revaccination study did not report a protective benefit. More recently the potential benefit of revaccinating adolescents with BCG has been reviewed and thought to be both cost effective and efficacious in some settings. Recently, in a prevention-of-infection Phase 2 trial of the novel vaccine candidate H4:IC31 conducted in South African adolescents, revaccination with BCG significantly reduced TB infections. Revaccination is not associated with an increased risk of adverse events, and there may be beneficial heterologous effects. Prevention of infection (POI) candidates and studies are most appropriate in paediatric cohorots. A number of promising candidates are progressing through vaccine pipeline, including VPM1002, M72 and H4:IC31. Recently, the infant trial of the MVA85A candidate, which conclusively failed to demonstrate added protection to that provided by newborn BCG vaccination, taught invaluable lessons about conduct of infant vaccine trials, pre-clinical animal models, community engagement, endpoint determination, and biomarkers of risk for TB.

O167 Triumphs, trials and tribulations of influenza vaccines

Maria Zambon[1]

[1]*Public Health England, United Kingdom*

Background: Over 14 million individuals are vaccinated against influenza in the UK every year. Vaccine campaigns from winter of 18/19 onwards will involve increasing diversity of vaccines. Different population segments receive different vaccines, making the landscape of influenza vaccines complex.

Objectives: Despite successes at increasing vaccine coverage and improvements in surveillance to detect variations in circulating strains, there remain significant challenges in (1) predicting which are the best virus strains to use for seasonal influenza vaccines and (2) optimising the effectiveness of current vaccines used in children and the elderly.

Methods: Strengths and limitations of seasonal influenza vaccines and progress towards universal influenza vaccines will be discussed.

Results: Making best use of the available vaccines is an important public health measure

O168 Dynamics and molecular basis of bacterial host adaptation

Ross Fitzgerald[1]

[1]*University of Edinburgh, The Roslin Institute, Edinburgh, United Kingdom*

Background: The capacity for some pathogens to adapt to different host-species provides opportunities for expansion into new host populations. *Staphylococcus aureus* is a major human pathogen that also causes economically important infections of livestock.

Objectives: We have been investigating the evolutionary history of *S. aureus* clones associated with different host species, the dynamics of host-switching events and the molecular basis for host-adaptation.

Methods: We have used a combination of time-scaled phylogenetics, comparative genomics of >800 isolates, functional analysis and experimental infections.

Results: We have identified ancient and recent host-switching events leading to the emergence of endemic and epidemic clones in humans and livestock. Our analyses have resulted in the identification of specific mutations and mobile genetic elements that have contributed to the capacity to infect different hosts.

O169 Genome evolution and adaptation of Achromobacter xylosoxidans within the airways of cystic fibrosis patients

Migle Gabrielaite[1], Helle Krogh Johansen[2], Rasmus Marvig[1]

[1]*Copenhagen University Hospital, Center for Genomic Medicine, Copenhagen, Denmark*
[2]*Copenhagen University Hospital, Department of Clinical Microbiology, Copenhagen, Denmark*

Background: Airway infections from opportunistic bacterial pathogens are a major cause of morbidity and mortality in cystic fibrosis (CF) patients, and within-host genetic adaptation has been hypothesized to play a role in these infections. Among the CF pathogens the role of within-host genetic adaptation of *Achromobacter xylosoxidans* remains highly unknown.

Objectives: We aim to analyze genome sequences of clinical isolates of *A. xylosoxidans* to better understand population genomics, within-host microevolution, mutational mechanisms, genetic adaptation and elucidate potential transmission events between CF patients.

Methods: We analyzed genome sequences of 103 clinical isolates of *A. xylosoxidans* collected longitudinally from 50 CF patients to establish a catalogue of genomic differences among the isolates. This included an analysis of the gene content of each isolate and an identification of small mutational changes within genes.

Results: We found that the population of *A. xylosoxidans* was genetically divided into four major groups that each encompassed a number of genetically distinct clone types (Fig. 1). Different CF patients were not infected by the same clone type; however, individual patients were observed to be infected with the same clone type over time. We found that the same genes were often mutated in persisting clonal lineages, indicating genetic adaptation. Furthermore, we determined the relative contribution of small and large genomic alterations, respectively, to the generation of the within-host genetic diversity. Overall, this analysis increases our understanding of bacterial adaptation to the host and facilitates comparative studies to other CF pathogens.

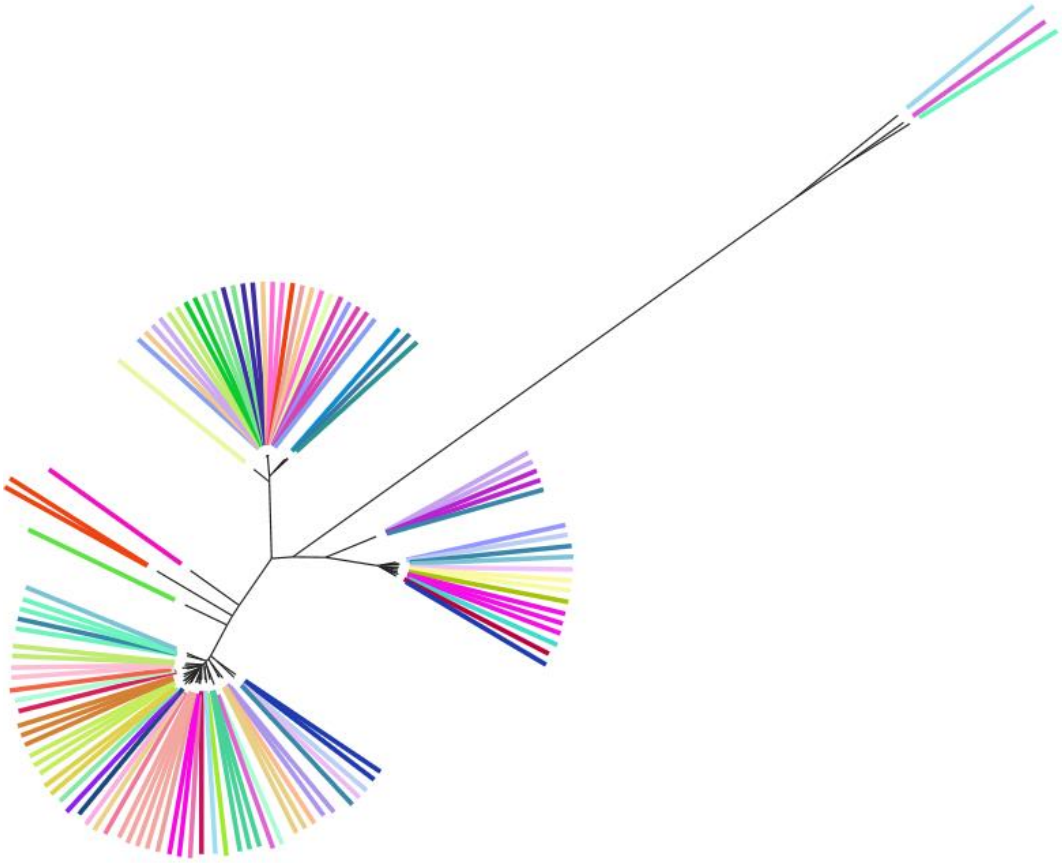


Figure 1. Phylogenetic tree of 103 *Achromobacter xylosoxidans* isolates. The phylogenetic tree is based on SNPs in the core genome.

O170 The role of the mobilome in the fall and rise of group B Streptococcus in cattle

Chiara Crestani[1], Mark Holmes[2], Samantha Lycett[3], Taya Forde[1], Karin Persson Waller[4], Ruth Zadoks[1]

[1]*University of Glasgow, Institute of Biodiversity Animal Health and Comparative Medicine, Glasgow, United Kingdom*
[2]*University of Cambridge, Department of Veterinary Medicine, Cambridge, United Kingdom*
[3]*University of Edinburgh, The Roslin Institute, Edinburgh, United Kingdom*
[4]*National Veterinary Institute, SVA, Travvägen, Uppsala, Sweden*

Background: *Streptococcus agalactiae,* also known as group B *Streptococcus* (GBS), is a pathogen of humans and cattle, in which it is responsible for carriage or invasive disease and subclinical mastitis, respectively. Due to the successful management practices on dairy farms, GBS disappeared from the Swedish cattle population between the early '80s to the late '90s. GBS was thought to consist of distinct host-specific subpopulations. Recent studies have shown that human and cattle subpopulations overlap, with only a few host-specific sequence types (ST). The ability of a strain to adapt to different host species may be linked with the acquisition of mobile genetic elements (MGE).

Objectives: Exploring the differences in GBS cattle population pre- and post-eradication, with a focus on the possible role of the mobilome in the evolution of these strains.

Methods: GBS isolates from milk (n= 113) were sequenced with Illumina MiSeq. Reads were filtered, trimmed with ConDeTri and assembled with SPAdes. Assemblies were evaluated with Quast, a core genome alignment was obtained with RAxML and displayed both as tree (iTOL) and network (Splitstree). Prokka and Roary were used for sequence annotation and pangenome construction.

Results: Network analysis revealed the presence of five main clades: two consisted of cattle-specific ST, three comprised ST originating from both cattle and humans. One of the clades was detected only pre-'80s, two only post-'90s and two were present in both periods. Isolates from the pre-eradication era were all tetracycline sensitive, whereas 53% of the post-'90s isolates harboured one or more tetracycline resistance genes.

O171 The evolution of the animal-adapted Mycobacterium tuberculosis complex

Daniela Brites[1;2], Chloé Loiseau[1;2], Fabrizio Menardo[1;2], Sebastien Gagneux[1;2]

[1]*Swiss Tropical and Public Health Institute*
[2]*University of Basel*

Background: Tuberculosis (TB) affects humans and other animals and is caused by bacteria from the *Mycobacterium tuberculosis* complex (MTBC). Unlike in humans the ecology and the evolution of the animal-adapted MTBC groups are not well understood.

Objectives: Provide a robust phylogenetic framework for the animal adapted-MTBC that can help us understanding major host transitions and the evolution of host-pathogen specificity within the MTBC.

Methods: We have gathered 529 genomes of animal-adapted MTBC after screening 12'886 publicly available MTBC genomes and newly sequenced 17 animal-adapted MTBC strains. We use comparative genomics, phylogenetic reconstructions, molecular dating and population genetics to get insights into the evolutionary history of the animal-adapted MTBC.

Results: We confirm that the animal-adapted MTBC members are paraphyletic; some are members more closely related to the human-adapted *Mycobacterium africanum* Lineage 6 than to other animal-adapted strains. Nucleotide diversity was not positively correlated with host phylogenetic distances, suggesting that host tropism is driven more by contact rates and demographic aspects of the host population than by host relatedness. Furthermore, four main animal-adapted MTBC clades that might correspond to main host shifts were identified. We hypothesize that *M. bovis* and *M. orygis*, reflect independent speciation events from a common ancestor into different cattle populations. Furthermore, genetic diversity estimates of *M. bovis* and *M. orygis* suggest that much of the diversity observed worldwide has originated in East Africa and South Asia, respectively. We combine molecular dating with the history of cattle domestication to provide an evolutionary scenario for the evolution of bovine TB.

O172 Prokaryotic Rab GTPases: a unique host adaptation tool of Legionella

Carmen Buchrieser[1], Sonia Mondino[1], Laura Gomez Valero[1]

[1]*Institut Pasteur, Genomes and Genetics, Paris, France*

Background: *Legionella* are environmental bacteria that replicate inside aquatic protozoa but are also able to infect humans where they replicate in alveolar macrophages and cause a severe pneumonia. *Legionella* host adaptation evolved through interdomain horizontal gene transfer (HGT) of eukaryotic functions

Objectives: We aimed to identify proteins allowing host adaptation of *Legionella* and thereby also allowing these bacteria to cause disease in humans.

Methods: We sequenced the genomes of 80 *Legionella* strains belonging to 58 species and analyzed them by using comparative genomics methods and phylogenetic analyses to identify proteins predicted to play a role in host pathogen interactions. The identified proteins were analysed experimentally.

Results: We identified 184 small GTPases of which 104 could be classified with a very high confidence as Rab, Ras or Rho like proteins (34 Ras, 71 Rab and one Rho domain). Blastp analysis (NCBI database) revealed that 149 of the 184 small GTPases of *Legionella* were exclusively present in *Legionella* and eukaryotic organisms. A subset of Rab proteins carried additional domains (U-box, ankyrin, F-box). Phylogenetic analysis of 16 Rab proteins present in eight different *Legionella* species showed that the Rab domains were acquired by HGT, mainly from protozoa. To substantiate that these proteins may act in the host cell, we undertook translocation assays for 16 Rab like proteins confirming their translocation. Detailed analyses of one of these Rab proteins, confirmed that it has indeed GTPase activity. Thus, secretion of prokaryotic Rab GTPases are a new way of host adaptation employed by *Legionella.*

O173 An essential mycolate remodeling program for mycobacterial host adaptation

Rebeca Bailo[1], Eliza J.R. Peterson[2], Min Pan[2], Charlotte Cooper[1], Nitin Baliga[2], Apoorva Bhatt[1]

[1]*Institute of Microbiology and Infection, School of Biosciences, University of Birmingham, Birmingham, United Kingdom*
[2]*Institute for Systems Biology, Seattle, United States*

Background: Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (Mtb), was one of the top 10 causes of death worldwide in 2017. Mycolic acids (MA) are distinct long-chain fatty acids found in the cells wall of mycobacteria including Mtb. MA are required for viability as they play important roles in the bacterial physiology, permeability, virulence and biofilm formation. Understanding MA biosynthesis and its regulation in Mtb and their role in the pathogenesis of active and latent infection is critical for the development of new vaccines and anti-TB drugs.

Objectives: Our aim was the identification of transcription factors (TF) that regulate MA biosynthesis in response to environmental cues including those that exist during infection.

Methods: Gene regulatory influence network (EGRIN) model of Mtb was used to identify MadR, a TF potentially regulating genes *desA1* and *desA2* involved in MA desaturation. To confirm the repression of *desA1/A2*, promoter binding was shown by Chip-Seq. Besides, the viability of the inducible TF-overexpression strain was checked on solid media, and its lipid profile was analysed by TLC. The role of MadR and *desA1/A2* during infection was also assessed by monitoring expression dynamics during Mtb infection of murine bone marrow-derived macrophages (BMDMs) using Path-Seq, a new method used to obtain global gene expression of both host and pathogen during infection.

Results: Using Path-seq and regulatory network analyses we have discovered that MadR transcriptionally modulates two mycolic acid desaturases *desA1/A2* to initially promote cell wall remodelling upon *in vitro* macrophage infection and, subsequently, reduces mycolate biosynthesis upon entering dormancy.

O174 Fitness and epistasis in the development of vancomycin-intermediate Staphylococcus aureus

Anaëlle Fait[1], Martin Vestergaard[1], Paal Skytt Andersen[2], Hanne Ingmer[1]

[1]University of Copenhagen, Department of Veterinary and Animal Sciences, Frederiksberg, Denmark
[2]Statens Serum Institut, Microbiology, Parasitology and Virology Department, Copenhagen S, Denmark

Background: Vancomycin is a last-resort antibiotic used against methicillin-resistant *Staphylococcus aureus* (MRSA), however, *S. aureus* strains with decreased susceptibility to vancomycin (vancomycin-intermediate *S. aureus* VISA) have been spreading during the past 20 years and are associated with severe infections and treatment failure. VISA strains harbor diverse and complex resistance mechanisms. They develop via the stepwise accumulation of mutations conferring mostly low-level resistance, resulting in tolerant phenotypes difficult to detect in the clinic. Key questions are if particular mutational events predispose rapid VISA evolution or evolution to higher resistance levels and if such events can be prevented.

Objectives: Examining the role of fitness and epistasis in the development of VISA to identify mutational events that promote rapid evolution or evolution to higher resistance levels.

Methods: We grew independent cultures of MRSA on increasing concentrations of vancomycin and saved culture samples at each passaging to track the phenotypic and genetic changes occurring chronologically during VISA development. Chronology in acquisition of mutations gives us an important insight into the role of non-obvious resistance genes that, while not necessarily increasing antibiotic resistance, promote the further development of VISA.

Results: We find that VISA strains develop by distinct evolutionary pathways deriving from a common ancestor, and show that vancomycin tolerance can arise at different pace and to different levels depending on early mutational events.

By characterizing early determinants that promote VISA development, our work can help implement guidelines for a better management of vancomycin use in the clinic, and reduce further resistance development in MRSA.

O175 Baterial and protozoal dynamics upon thawing and freezing of an active layer permafrost soil

Morten Schostag[1], Anders Priemé[2], Jacquiod Samuel[2], Jakob Russel[2], Flemming Ekelund[2], Carsten Suhr Jacobsen[3]

[1]*GEUS, Department of Geochemistry, København K, Denmark*
[2]*University of Copenhagen, Department of Biology, Copenhagen N, Denmark*
[3]*Aarhus University, Department of environmental science, Roskilde, Denmark*

Background: The active layer of soil overlaying permafrost in the Arctic is subjected to annual changes in temperature and soil chemistry, which we hypothesize to affect the overall soil microbial community.

Objectives: We investigated changes in soil microorganisms at different temperatures during warming and freezing of active layer soil from Svalbard.

Methods: Soil community data were obtained by direct shotgun sequencing of total extracted RNA.

Results: No changes in soil microbial communities where detected when warming from -10°C to -2°C or when freezing from -2°C to -10°C. In contrast, within a few days we observed changes when warming from -2°C to +2°C with decrease in fungal rRNA and increase in several OTUs belonging to Gemmatimonadetes, Bacteroidetes and Betaproteobacteria. Even more substantial changes occurred when incubating at 2°C for 16 days, with declines in total fungal potential activity and decreases in oligotrophic members from Actinobacteria and Acidobacteria. Additionally, we detected an increase in transcriptome sequences of bacterial phyla Bacteriodetes, Firmicutes, Betaproteobacteria and Gammaproteobacteria - collectively presumed to be copiotrophic. Furthermore, we detected an increase in putative bacterivorous heterotrophic flagellates, likely due to predation upon the bacterial community via grazing. Althrough this grazing activity may explain relatively large changes in the bacterial community composition, no changes in total 16S rRNA gene copy number were observed and total RNA level remained stable during the incubation. Together these results are showing the first comprehensive ecological evaluation across prokaryotic and eukaryotic microbial communities on thawing and freezing of soil by application of the TotalRNA technique.

O176 Shades of Brown: how ice algae are melting the Greenland ice sheet

Alexandre Anesio[1], Christopher Wiliamson[2], Martyn Tranter[2], Team Black and Bloom[3]

[1]*Aarhus University, Department of Environmental Science, Roskilde, Denmark*
[2]*University of Bristol, School of Geographical Sciences, Bristol, United Kingdom*
[3]*Universities of Bristol, Sheffield and Leeds, United Kingdom*

Background: It is now recognised that large expanses of ice in the polar and alpine regions are inhabited by active microbial communities forming one of the biomes of Earth. Microbes on glaciers and ice sheets are diverse, play an important role in the cycling of nutrients and can also modify the physical environment they live. For instance, microbial processes at the surface of the ice can lead to the accumulation of dissolved and particulate organic carbon. The accumulation of cells often seen at the surface of the ice results in 'biological darkening' of glacier surfaces. The ice algal community on the Greenland ice sheet (GrIS) is dominated
by *Mesotaenium berggrenii* and *Ancylonema nordenkioldii,* and the presence of these algae reduces the albedo of the ice surface, mostly due to a brown-purple purpurogallin-like pigment.

Objectives: Here, we will show that ice algae are the dominant albedo reducing particulate in the west side of the Greenland ice sheet and demonstrate that light stress is key in stimulating the production of phenolic-based pigments in ice algae.

Methods: We used a combination of pulse amplitude modulation fluorescence, primary productivity, counts combined with pigment and albedo quantification and remote sensing information.

Results: We estimate albedo reduction of the ice between 12% and 21%, depending on the algal cell abundances. Such darkening increases the amount of incident shortwave radiation available for ice ablation and is a clear contributing element to glacier thinning and wastage.

O177 Circumpolar microbiome patterns as vulnerability determinants of subducted carbon in arctic permafrost soils

Tim Urich[1]

[1]*Institute of Microbiology, Greifswald, Germany*

Background: Arctic permafrost soils contain more than 1300 Pg of organic carbon (OC), about half of the global soil OC and twice as much C as is currently contained in the atmosphere. Besides temperature and other physicochemical parameters like moisture and oxygen, the soil microbiome (including bacteria and fungi) is thought to be a crucial factor for SOM vulnerability to decomposition in these cryoturbated soils. There is now solid evidence that C loss from arctic top soils is likely to occur. However, there is limited data on the sensitivity of C stocks in deeper soil horizons, although the majority of C resides therein.

Objectives: We aimed to identify if global patterns in the permafrost soil microbiome associated with OC decomposition can be identified.

Methods: We investigated the abundance, community composition and activity of prokaryotes and fungi in > 500 soil samples from the Siberian and Canadian Arctic and Greenland.

Results: Here we show that a distinct microbiome pattern is associated with buried C and that small scale microbial ecology regulates global biogeochemical processes in permafrost soils. We identified a circumpolar pattern in the microbiomes, with low abundance of fungi in buried C layers, following a predictable depth-dependent proxy. In contrast, bacterial abundances were controlled by OC content. Incubation experiments showed that both groups positively correlated with C mineralisation. We thus show that microbiome members crucial for SOM decomposition are globally controlled by different factors resulting in distinct microbiomes in buried OM horizons that ultimately regulate SOM vulnerability to warming.

O178 Impact of global warming on permafrost soil microbiomes and their potential for greenhouse gas production

Janet Jansson[1]

[1]*Pacific Northwest National Laboratory, Biological Sciences Division, Richland, United States*

Background: The Arctic is one of the most climate-sensitive regions on Earth where average temperatures are increasing at nearly twice the global rate. This is resulting in dramatic changes in the landscape, including permafrost thaw, increased decomposition of organic carbon by permafrost soil microorganisms, and release of greenhouse gases.

Objectives: We aimed to determine the composition and activity of permafrost microbes before and after thaw and how changes in landscape hydrology influence the potential for greenhouse gas production by the permafrost soil microbiome.

Methods: We used a multi-omics approach to determine the composition and functions of the permafrost soil microbiome before and after thaw. We also used metagenome sequencing to obtain complete to near-complete genomes from arctic soils, including from previously uncharacterized taxa.

Results: Our results revealed that some taxa were actively transcribing and translating genes in permafrost, with the surprising finding that iron reduction was highly expressed in the proteome data. With depth into the permafrost layer, the microbial community composition changed towards more resilient species that are capable of long-term survival under cold, nutrient poor conditions; mainly members of the actinobacteria phylum. Metagenome sequencing demonstrated that the microbial compositions and their functional potential in permafrost are dissimilar from those in the active layer and that there is a rapid shift upon thaw. Investigation of binned genomes from metagenomes provided clues to how permafrost microbes are genetically equipped to survive in arctic soils and their potential to respond to changing environmental conditions.

O179 Microbial warfare mediated by the Type VI secretion system

Sarah Coulthurst[1]

[1]*University of Dundee, School of Life Sciences, Dundee, United Kingdom*

Protein secretion systems are specialised macromolecular machines used to translocate specific proteins out of the bacterial cell, where they can be either released to the external environment or injected into other cells. Secretion systems, and the diverse proteins they secrete, mediate the interaction of bacterial cells with the environment, eukaryotic cells or other bacteria. The Type VI secretion system (T6SS) is a key weapon in the competitiveness and virulence of very many Gram-negative bacteria. Whilst some T6SSs are 'anti-eukaryotic', used to target host cells as classical virulence factors, the majority appear to be 'anti-bacterial', used to efficiently kill rival bacterial cells and provide a competitive advantage in a variety of polymicrobial niches. T6SSs deliver multiple, diverse toxins ('effectors') directly into target cells by a contraction-based 'firing' mechanism. We have used the potent anti-bacterial T6SS of the opportunistic pathogen *Serratia marcescens* as a model to study the mechanisms and consequences of T6SS effector delivery into competitors. This has revealed a varied portfolio of effector toxins and cognate immunity proteins, used for several forms of inter-microbial competition. In particular, we have discovered that the *S. marcescens* T6SS is not only able to target bacterial competitors, but also to deploy anti-fungal effector proteins against microbial fungi. These findings make a key contribution to the growing appreciation that the role of the T6SS in shaping polymicrobial communities is much broader than initially thought.

O180 Streptomyces coelicolor extracellular vesicles carry a complex and unexpected set of proteins

Teresa Faddetta[1], Giovanni Renzone[2], Francesco Amato[3], Carmelo Miccichè[3], Emilio Rimini[1], Roberto Caruana[4], Laura Modica de Mohac[5], Giorgio Nasillo[4], Gianpiero Buscarino[3], Simonpietro Agnello[3], Mariano Licciardi[1], Luigi Botta[6], Antonio Palumbo Piccionello[1], Andrea Scaloni[2], Giuseppe Gallo[1], Anna Maria Puglia[1]

[1]University of Palermo , Department of Biological Chemical and Pharmaceutical Sciences and Technology (STEBICEF), Palermo, Italy
[2]CNR (Consiglio Nazionale delle Ricerche), Proteomic and Mass Spectrometry Laboratory, ISPAAM, Napoli, Italy
[3]University of Palermo , Department of Physics and Chemistry, Palermo, Italy
[4]University of Palermo , Advanced Technologies Network (ATeN Center), Palermo, Italy
[5]University of Palermo , Department of Internal Medicine and Medical Specialties (DIMIS), Italy
[6]University of Palermo , Department of Civil, Environmental, Aerospace, Materials Engineering (DICAM), Palermo, Italy

Background: Extracellular vesicles (EVs) are produced by cells in all domains of life. EVs carry macromolecules and metabolites and are involved in cell communication, competition and survival. EVs have been characterized in many Gram-negative bacteria and, more recently, in several Gram-positive bacteria.

Streptomyces coelicolor is a model organism for the study of *Streptomyces*, a genus of Gram-positive bacteria that undergoes a complex life cycle characterized by a differentiation program, involving the formation of vegetative and aerial mycelium and spores, and by the production of an enormous repertoire of bioactive metabolites and extracellular enzymes.

Objectives: Characterization of *S. coelicolor* EV protein cargo.

Methods:

- Isolation and purification of EVs by density gradient ultracentrifugation;
- Transmission Electron Microscopy, Scanning Electron Microscopy, Atomic-Force Microscopy, Raman microscopy and Dynamic Light Scattering analyses;
- Proteomic analysis based on Liquid Chromatography Tandem-Mass Spectrometry.

Results: *S. coelicolor* produces EVs with sizes ranging between 100 and 200 nm. Vesicular cargo includes proteins, nucleic acids and the antibiotic actinorhodin. In particular, EVs carry a complex set of proteins involved in cell metabolism, molecular processing and transport. Interestingly, some proteins such as cytochromes, bacterioferritin and the spore-associated SapA protein are inside vesicles and some other proteins, such as ribosomal and stress response protein, are associated to EV surface as suggested by protease accessibility assay coupled with proteomics. Many of these proteins are involved in physiological and morphological differentiation.

Therefore, this study may shed light on the role of the extracellular vesicles in *Streptomyces* physiology and development.

O181 Post-secretional activation of extracellular proteases by quorum sensing in Pseudomonas aeruginosa

Xihui Li[1], Joon-Hee Lee[1]

[1]*Pusan National University, College of Pharmacy, Department of Pharmacy, Busan, Korea, Rep. of South*

Background: *Pseudomonas aeruginosa* secretes many extracellular proteases that are implicated in *P. aeruginosa* pathogenesis. Among them, protease IV (PIV), LasA, and LasB are quorum sensing (QS)-regulated in transcription level. Our previous research found that PIV is post-secretionally activated by LasB-mediated extracellular degradation of the propeptide in QS-dependent manner.

Objectives: We found that LasA overexpressed in QS mutant has a severe reduction in its activity despite high level expression and secretion. We intended to elucidate the underlying mechanism in this post-secretional activation of LasA by QS.

Methods: SDS-PAGE analysis showed that most LasA in QS mutant was in its non-processed form (proLasA), suggesting that the processing of LasA occurs in extracellular space by a QS-dependent factor. Our results demonstrated that LasB and PIV cleave and degrade the propeptide of LasA to make mature and active LasA. Deletion of *lasB* remarkably reduced the activity of both PIV and LasA, indicating that LasB activates both PIV and LasA. LasA activity was reduced more than 60% in *Δpiv*, suggesting that PIV more directly contributes to the extracellular processing of LasA. Our results indicated that LasB, PIV, and LasA are not only transcriptionally controlled by QS, but also activated post-secretionally by each other in a QS-dependent manner.

Results: We suggest the cascade activation of three extracellular proteases, LasB, PIV, and LasA in extracellular space after secretion. LasB first activates PIV by degrading the PIV propeptide, and then the activated PIV activates LasA by degrading the LasA propeptide. LasB also can partly activate LasA.

O182 The Pseudomonas aeruginosa T6SS-VgrG1b tip is capped by a PAAR protein eliciting DNA damage to bacterial competitors

Luke P. Allsopp[1;2], Panayiota Pissaridou[2], Sarah Wettstadt[2], Sophie A. Howard[2], Despoina A.I. Mavridou[2], Alain Filloux[2]

[1]*Imperial College London, Faculty of Medicine, National Heart and Lung Institute, LONDON, United Kingdom*
[2]*Imperial College London, Department of Life Sciences, MRC Centre for Molecular Bacteriology and Infection, LONDON, United Kingdom*

Background: The type VI secretion system (T6SS) is a molecular nanomachine which delivers potent toxins directly into host cells or bacterial competitors. *Pseudomonas aeruginosa* encodes three T6SS gene clusters and multiple *vgrG* gene islands which encode for different tips of the T6SS.

Objectives: To characterised the *vgrG1b* operon.

Methods: We used bioinformatic tools to predict the function of the genes encoded in the *vgrG1b* island. Subsequently, we use a range of molecular microbial approaches such as cloning, growth assays, western blot analysis, molecular modeling, dot blot analysis and T6SS killing assays to characterise this cluster.

Results: The *vgrG1b* cluster encodes two genes we name *tse7* and *tsi7* which are striking divergent compared to the other genes of the cluster. We identified that *tse7* encodes a Tox-GHH2 nuclease domain at its C-terminus. Expression of Tse7 in bacteria induces the SOS response, leads to growth arrest and ultimately results in DNA degradation. Additionally, we show that the N-terminus of Tse7 encodes a PAAR-like domain. This PAAR domain facilitates coupling of Tse7 onto the H1-T6SS VgrG1b timer for delivery into prey bacteria. We could demonstrate that a *P. aeruginosa* strain deleted for the *vgrG1b* operon, including *tsi7*, becomes susceptible to inter-bacterial Tse7 mediated killing. Finally we identify, Tsi7, the corresponding immunity protein, which protects the producer from Tse7 via direct protein interaction. This study highlights the specific fitting of toxic effectors onto the tip of the T6SS, the strain diversity of T6SS effectors and immunity proteins which suggests a role in inter-strain competition.

O183 Insights into the autotransporter process of a trimeric autotransporter, Yersinia Adhesin A (YadA)

Daniel Hatlem[1], Nandini Chauhan[1], Marcella Orwick-Rydmark[1], Kenneth Schneider[1], Matthias Floetenmeyer[2], Barth van Rossum[3], Jack Leo[1], Dirk Linke[1]

[1]University of Oslo, Department of Biosciences, Oslo, Norway
[2]The University of Queensland, The Centre for Microscopy and Microanalysis, Saint Lucia, Australia
[3]Forschungsinstitut für Molekulare Pharmakologie, Department of NMR-Supported Structural Biology, Berlin, Germany

Background: Trimeric autotransporter adhesins (TAAs) are a subset of a larger protein family called the type V secretion systems. They are localized on the cell surface of Gram-negative bacteria, function as mediators of attachment to inorganic surfaces and host cells, and thus include important virulence factors. *Yersinia* adhesin A (YadA) from *Yersinia enterocolitica* is a prototypical TAA that is used extensively to study the structure and function of the type V secretion system. A solid-state NMR study of the membrane anchor domain of YadA previously revealed a flexible stretch of small residues, termed the ASSA region, which links the membrane anchor to the stalk domain. This region is thought to form a hairpin intermediate that is essential for the initiation of the autotransporter process.

Objectives: In this work, we set out to investigate whether TAAs initiate transport via a hairpin intermediate in a mechanism comparable to other autotransporters, using YadA as our established model system.

Methods: SpyTag-SpyCatcher assay, transmission electron microscopy (TEM), Fourier-transform infrared (FTIR) spectroscopy, autoaggregation assay

Results: We present evidence that single amino acid proline substitutions in the ASSA region produce two different conformers of the membrane anchor domain of YadA; one with the N-termini facing the extracellular surface, and a second with the N-termini located in the periplasm. We propose that TAAs adopt a hairpin intermediate during secretion, as has been shown before for other subtypes of the type V secretion systems

O184 Modulation of the activity of the ClpCP protease in Bacillus subtilis by a phage protein

Nancy Mulvenna[1], Sophie Nicod[2], Lynn Burchell[1], Ingo Hantke[3], Ramesh Wigneshwararaj[1]

[1]*CMBI Imperial College London, Medicine, London,*
[2]*University of Sussex, Biochemistry, Brighton, United Kingdom*
[3]*Leibniz University Hannover, Microbiology, Hanover, Germany*

Background: Much like eukaryotic and archaeal viruses, which derail the host's cellular processes to ensure viral replication, phages have evolved complex strategies to take over their bacterial hosts. Many phages encode specialised small proteins, which we refer to has 'host acquisition factors' (HAFs) that modulate, inhibit or repurpose essential bacterial processes to promote phage progeny development.

Objectives: Identify HAFs in phages.

Methods: Molecular microbiology, mass spectrometry

Results: We describe the identification of a HAF, called Gp53, encoded by the *Bacillus subtilis* infecting phage SPO1. We reveal that Gp53 interacts with the ClpC ATPase protein of the ClpCP protease of *B. subtilis*. We demonstrate that Gp53 stimulates the ATPase activity of ClpC and modulates the proteome of *B. subtilis*. Since the ClpCP protease of *B. subtilis* requires the binding of so called 'adaptor' proteins for specific substrate recognition for proteolysis, our results suggest that Gp53 functions as a phage-encoded 'adaptor' of the ClpCP protease, which modulates the proteome of the infected bacterial cell to ensure optimal requirements for SPO1 replication are met.

O185 Overexpression of tRNAGly affects the solubility of a cyclin altering growth of Schizosaccaromyces pombe

Omar Orellana[1], Loreto Arias[2], Sandra Moreira[2], Assaf Katz[2]

[1]Universidad de Chile Facultad de Medicina, Programa de Biología Celular y Molecular, ICBM, Santiago, Chile
[2]Universidad de Chile, Facultad de Medicina, Programa de Biología Celular y Molecular, ICBM, Santiago, Chile

Background: Under stress, gene expression can be affected by changes in mRNA and protein levels. Under oxidative stress certain yeast proteins, such as Cdc13, are specifically regulated at the translational level. Cdc13 is a cyclin essential for the G2/Mitosis cell cycle transition. Under oxidative stress, the level of this protein increases without changing mRNA level. Changes in tRNA levels might explain the effect on translation of Cdc13 mRNA.

Objectives: The purpose of this work was to determine whether increasing tRNA levels that decode rare codons in cdc13 mRNA, affect the expression and folding of the Cdc13 and/or cell growth in Schizosaccharomyces pombe.

Methods: Northern blot to asses tRNA levels of H_2O_2-induced oxidative stress cells and overexpressed tRNA genes. Microscopy assessment of cell phenotype. PAGE to asses protein aggregation.

Results: H_2O_2-induced oxidative stress resulted in an increased level of $tRNA^{Gly}_{UCC}$ and $tRNA^{Arg}_{UCU}$ while $tRNA^{Thr}_{UGU}$ did not change. Because $tRNA^{Gly}_{UCC}$ has a low gene dosage (decoding a rare codon), we studied the effect of the overexpression on this tRNA in the expression and solubility of Cdc13. Overexpression of the $tRNA^{Gly}_{UCC}$ gene increased aggregation of Cdc13 but did not affect the mRNA and protein levels. Additionally, overexpression of this tRNA resulted in elongated cells. However, overexpression of the high gene dosage isoacceptor $tRNA^{Gly}_{GCC}$, decoding an optimal codon, did not generate the elongated cell phenotype. In conclusion, these results revealed that overexpression of the $tRNA^{Gly}_{UCC}$ affected the solubility of cdc13. This effect could generate the elongated phenotype as a defect of the cell cycle.

O186 The long isoform of CobB sirtuin is acetylated by the YiaC N-alpha-acetyltransferase

Anastacia Parks[1], Jorge Escalante-Semerena[1]

[1]*University of Georgia, Microbiology, Athens, United States*

Background: Protein *N*-alpha acetylation is common in eukaryotes, but not in prokaryotes. *N*-alpha acetylation can alter protein folding, binding interactions, and degradation. In prokaryotes, only bacterial ribosomal proteins are *N*-terminally acetylated. The genome of *Salmonella enterica* subsp. *enterica* sv Typhimurium strain LT2 (hereafter *S. enterica*) contains one sirtuin deacylase-encoding gene, *cobB* that synthesizes two biologically active isoforms, $CobB_{S(hort)}$ and $CobB_{L(ong)}$, which differ in size by a 37-residue *N*-terminal extension. Here we report that $CobB_L$ isoform of *S. enterica* is acetylated by the acetyltransferase, YiaC. To our knowledge, this is the first instance of *N*-terminal acetylation of a bacterial non-ribosomal protein.

Objectives: To characterize the *N*-terminal acetylation of the sirtuin deacylase $CobB_L$ by the acetyltransferase YiaC in *S. enterica*.

Methods: We show that radiolabeled acetyl moiety of acetyl-coenzyme A (AcCoA) is transferred to $CobB_L$ by YiaC. LC/MS/MS analysis confirmed *N*-terminal acetylation of $CobB_L$. Additional support was obtained *in vitro* using [1-^{14}C]-acetyl-CoA, synthetic peptides and lysine $CobB_L$ variants. Acetylation by YiaC affected $CobB_L$ function *in vivo* during growth of *S. enterica* using acetate and *in vitro* with sirtuin activity assays.

Results: Our *in vitro* studies demonstrated that $CobB_L$ but not $CobB_S$ was acetylated, and that YiaC modified the α-amino group of the *N* terminus. This modification prevented growth of *S. enterica* when $CobB_L$ was required. We report the first finding of a posttranslational modification of a prokaryotic sirtuin. This discovery expands the understanding of *N*-terminal acetylation of prokaryotic proteins and the regulation of sirtuins.

O187 Cultivation, physiology, genomic, and exometabolomic study of previously uncultivated Chloroflexi lineage TK10 (TK17), a global inhabitant of geothermal springs and marine environments

Brian Hedlund[1], Enmin Zhou[1;2], Scott C. Thomas[1], Neeli Habib[2], Jian-Yu Jiao[2], Jonathan Covington[1], Dengxun Lai[1], Li-Xin Chen[2], Cale O. Seymour[1], Senthil Murugapiran[1], Jeremy A. Dodsworth[3], Chrisabelle R. Mefferd[1], Lian Liu[2], Wen-Dong Xian[2], Xiao-Yang Zhi[4], Katherine Louie[5], Benjamin P. Bowen[5], Trent R. Northen[5], Tanja Woyke[5], Wen-Jun Li[2]

[1]School of Life Sciences, University of Nevada, United States
[2]College of Ecology and Evolution, Sun Yat-Sen University, State Key Laboratory of Biocontrol and Guangdong Key Laboratory of Plant Resources, China
[3]California State University, Department of Biology, San Bernardino, California, United States
[4]Yunnan University, Yunnan Institute of Microbiology, Kunming, China
[5]Joint Genome Institute, Department of Energy, Walnut Creek, California, United States

Two novel thermophilic, aerobic, and heterotrophic bacteria, designated strain G233^T and YIM 72310^T, were isolated from sediments of Great Boiling Spring (GBS) in the US Great Basin, and Hamazui spring (HMZ) in Rehai National Park, China. Growth occurred at 50-65 ^{o}C (optimum 55-60 ^{o}C) and pH 6.0-8.5 (optimum pH 7.0). Based on 16S rRNA gene sequence analysis, the both strains belonged to the phylum *Chloroflexi*, although both were distant from the closest relative, *Caldilinea aerophila* DSM 14535^T (85.8 % nucleotide identity). Phylogenies inferred from the 16S rRNA gene and the bac120 marker gene set indicate that the two strains are the first cultivated representatives of the 'TK10' lineage, which may represent a novel class or order. Although both strains grew to high cell density on R2A, few compounds were used as sole carbon and energy sources; however, genomic predictions and exometabolomics profiles indicate a much broader heterotrophic repertoire for these organisms, including heterotrophic activity on a variety of phenolics, heterocycles, and lipids and lipid derivatives. We suggest these organisms may specialize in digesting complex extracellular material in photosynthetic mats at the fringes of geothermal springs from which they were isolated. On the basis of their unique phylogenetic placement and physiological characteristics, we propose the names *Planoflexus thermophilus* sp. nov. and *Planoflexus flavus* sp. nov. for G233^T and YIM 72310^T, respectively. Further investigation will guide the higher-level taxonomy of these isolates and related single-cell genomes and metagenome-assembled genomes from terrestrial geothermal springs and marine ecosystems.

O188 Taxonomic structure of the Acidobacteria as a reflection of different lifestyles and ecological preferences

Svetlana Dedysh[1]

[1]*Winogradsky Institute of Microbiology, Research Center of Biotechnology RAS, Moscow, Russian Federation*

Background: Acidobacteria is one of the globally distributed and phylogenetically diverse bacterial phyla. The currently explored diversity within this phylum is commonly addressed as corresponding to 26 major 16S rRNA gene sequence clades or subdivisions. Recently, these 26 subdivisions were assigned to 15 class-level units, five of which contain described members. *Acidobacteria*-affiliated 16S rRNA gene sequences are commonly retrieved from various environments in cultivation-independent studies. Vast majority of these studies, however, simply report the observed pattern of acidobacterial diversity in the target habitat based on the proportion of reads affiliated with different subdivisions. The functional roles of acidobacteria identified in these studies are only rarely discussed.

Objectives: This analysis was undertaken in order to examine the lifestyles and ecological preferences of acidobacteria from different taxonomic groups.

Methods: The analysis was made using a comprehensive set of 16S rRNA gene sequences from characterized members of this phylum and as-yet-uncultivated representatives. The available genome sequences and the functional capabilities of taxonomically described organisms were taken into account.

Results: The analysis revealed considerable phenotypic similarity of acidobacteria within particular orders and families. Thus, members of the orders *Acidobacteriales* and *Bryobacterales* are acidophilic/acidotolerant bacteria with the preference for the aerobic/microaerobic lifestyle. Cold-tolerance and hydrolytic capabilities determine their wide distribution in low-temperature terrestrial habitats rich in organic matter. The corresponding profiles of 'ecological fitness' could be determined for other taxonomic groups of acidobacteria as well. This linkage between the taxonomic affiliation and the potential functional capabilities could help interpreting the results of molecular diversity surveys.

O189 Metabolic versatility, complete ammonia oxidizers, and more: A new perspective on the nitrifying microorganisms

Holger Daims[1]

[1]*University of Vienna, Department of Microbiology and Ecosystem Science, Vienna, Austria*

Background: Nitrogen (N) is made bioavailable and recycled into the atmosphere by microorganisms in the biogeochemical N-cycle. A major N-cycle process is nitrification, the oxidation of ammonia via nitrite to nitrate. Nitrification is the main biological source of nitrate and indispensable for wastewater treatment. However, nitrification also causes N-loss from fertilized soils and contributes globally to nitrous oxide emissions. Traditionally, the nitrifying microbes were regarded as highly specialized organisms whose growth is tightly linked to aerobic nitrification. Moreover, a paradigm of N-cycle microbiology stated that ammonia and nitrite oxidation are catalyzed by different microorganisms in cooperation. For decades, nitrification research in microbiology and environmental engineering has relied on these firm assumptions.

Results: Recent studies in microbial ecology have revealed a much more complex ecophysiology of ammonia and nitrite oxidizers. Novel alternative metabolisms were identified in globally distributed nitrite-oxidizing bacteria (NOB) from the genera *Nitrospira* and *Nitrococcus*, which enable these NOB to utilize energy sources other than nitrite and grow independently of nitrification. Ammonia-oxidizing archaea were found to tap novel sources of ammonia, and even anaerobic energy metabolisms were identified in nitrifiers. Surprisingly, complete ammonia oxidizers (comammox) were discovered in the genus *Nitrospira*. Comammox catalyze full nitrification alone and are widely distributed in diverse ecosystems. Their physiological properties suggest that comammox could be important for nitrification management and the mitigation of greenhouse gas emissions in agriculture and sewage treatment. Altogether, these findings have turned over the traditional picture of nitrification and expanded our perspective of the, mostly poorly characterized, nitrifying microorganisms.

O190 Acetispirales, a new order of homoacetogenic deltaproteobacteria from insect guts

Dorothee Tegtmeier[1], Katharina Klomann[1], Anna Faber[1], Katja Meuser[1], Renate Radek[2], Thomas Heimerl[3], Vincent Hervé[1], Andreas Brune[1]

[1]Max Planck Institute for Terrestrial Microbiology, RG Insect Gut Microbiology and Symbiosis, Marburg, Germany
[2]Free University of Berlin, Institute of Biology/Zoology, Berlin, Germany
[3]Philipps-Universität Marburg, LOEWE Research Center for Synthetic Microbiology (SYNMIKRO), Marburg, Germany

Reductive acetogenesis from H_2 and CO_2 is an important process in termite guts. Unlike in other environments, it is not catalyzed by homoacetogenic *Firmicutes* but instead by a termite-specific lineage of spirochetes. Recent studies documented that the enzymes required for the Wood-Ljungdahl pathway are also present in the genome of '*Candidatus* Adiutrix intracellularis', an endosymbiont of termite gut flagellates that represents a deep-branching clade of *Deltaproteobacteria*, the Rs-K70 group. Although this group is widely distributed in intestinal tracts of termites and cockroaches, its members have not been cultured. We isolated the first representative of this group from cockroach guts. *Acetispira formosa* grows by reduction of CO_2 with hydrogen or formate and by the homoacetogenic fermentation of glucose and *N*-acetylglucosamine. Comparative analysis of the genomes of *A. formosa* and '*Ca.* Adiutrix intracellularis' and the draft genome of an uncultured strain from a higher termite reconstructed by metagenomic analysis revealed that the same set of genes is involved in reductive acetogenesis in all three organisms. The hydrogen-dependent CO_2 reductase, a key enzyme of the pathway, which was most likely acquired via lateral gene transfer, appears to be of clostridial origin. However, none of the genomes encode an energy-converting hydrogenase or an Rnf complex. Instead, they encode an 11-subunit complex that has been implicated in energy conservation of methyl-reducing methanogens, which suggests that energy metabolism in *Acetispirales* differs from that in homoacetogenic *Firmicutes*.

O191 Potential and mechanisms of methane production by deep subsurface microorganisms in oil and gas fields

Susumu Sakata[1]

[1]*Geological Survey of Japan, AIST, Tsukuba, Japan*

Background: Natural gas is the product of either thermal or microbial degradation of organic matter in the subsurface environments. The microbial gas is estimated to account for about 20%. It consists mostly of methane produced by subsurface methanogens. Methanogens are assumed to use very limited small compounds such as H_2+CO_2, acetate, and methyl compounds, which should be produced by thermal or microbial degradation of larger organic matter. However, details of such processes are yet to be verified.

Objectives: We aim to investigate how deep subsurface microorganisms in oil and gas fields convert kerogen and crude oil to methane and if methanogens can use methoxy groups abundant in coal.

Methods: We constructed microcosms mimicking subsurface environments in the fields of microbial gas and biodegraded oil, observed the methane production potential, and analyzed the microbial community structures. Also, we cultivated methanogens with various methoxylated aromatic compounds (MACs) and coal.

Results: A large amount of methane was produced in microcosms for the microbial gas fields, implying direct microbial degradation of kerogen. The predominance of *Alkalibaculum bacchi* and *Methanosaeta* species suggests acetogenesis from MACs followed by acetoclastic methanogenesis as among the reaction pathways. Conversion of toluene and other alkylbenzenes to methane was observed in microcosms for the biodegraded oil field. Our metagenomic analysis showed that Peptococcaceae bacterium and Ca. Atribacteria are involved in the degradation of aromatic hydrocarbons. Among Methanosarcinales methanogens, *Methermicoccus shengiensis* produced methane from >30 kinds of MACs. It also showed the activity of direct methane production from coal.

O192 Manuka Honey: Therapeutic potentials for cystic fibrosis infections

Aled Roberts[1], Rowena Jenkins[1]

[1]*Swansea University, Institute of Life Sciences, Swansea, United Kingdom*

Background: Many patients suffering with chronic infections, such as those diagnosed with Cystic Fibrosis (CF), are at a crossroads. Over time their infections become increasingly debilitating whilst the number of effective treatment options subsides due to increased occurrences of antimicrobial resistance. This coupled with a reduction of novel antimicrobials requires the identification of novel drug combinations, or the repurposing of current antimicrobials. Manuka honey, currently a topical antimicrobial agent could fulfil both these objectives.

Objectives: The main objectives of this study were to ascertain the effects of manuka honey on CF-associated pathogens, detect manuka honey-antibiotic combinations with increased efficacy, and to identify any potential consequences of pro-longed use (spontaneous generation of tolerant mutants).

Methods: Inhibitory efficacy of manuka honey alone and in combination with clinically relevant antibiotics was tested using gold standard methods. A specialised 14-day resistance training experiment, coupled with a 14-day recovery period, was used to identify tolerance towards prolonged use. Finally, inhibitory efficacy was tested against biofilms grown in a specialised *ex vivo* pig lung model.

Results: Manuka honey inhibited CF-associated pathogens alone, and in combination with antibiotics, however combination therapy yielded varying results (species and concentration dependent). Prolonged exposure to sub-inhibitory concentrations was not sufficient enough to sustain an increased tolerance towards manuka honey. Against biofilms, increased concentrations of manuka honey were required to induce log-fold reductions in cellular viability, a feat not observed with increased antibiotic concentrations, however combination therapy lead to instances of total inhibition.

O193 The effects of NX-AS-401 on Methicillin Resistant Staphylococcus aureus

Phillip Butterick[1;2], Rowena Jenkins[1], Daniel Neef[2]

[1]*Swansea University, Microbiology and Infectious Disease, Swansea, United Kingdom*
[2]*Neem Biotech, Abertillery, United Kingdom*

Background: Chronic infections brought about by antimicrobial resistant bacteria and the development of bacterial biofilms require innovative new drugs for treatment. NX-AS-401 represents a novel approach in development at Neem Biotech, designed to act as an anti-virulence drug preventing infection and biofilm formation while working to enhance the activity of current standard of care antibiotics.

Objectives: The aim of this research was to identify the effects NX-AS-401 has on Methicillin Resistant *Staphylococcus aureus* (MRSA) in terms of growth, virulence expression and biofilm formation.

Methods: Initial studies employed EUCAST broth microdilution methods to obtain Minimum Inhibitory/Bactericidal concentrations of eight strains of MRSA and were adapted for biofilm inhibition/disruption. Scanning Electron Microscopy (SEM) was used to investigate changes in cell morphology and biofilm structure. RT-qPCR determined changes in genetic expression in virulence and biofilm-associated genes. Interactions between NX-AS-401 lead compounds and antibiotics were determined using checkerboards.

Results: 128 ug/mL of NX-AS-401 inhibits the growth of planktonic MRSA and significantly disrupts established biofilms ($p=<0.05$). SEM showed a decrease in cell numbers in biofilms after treatment with NX-AS-401. RT-qPCR identified significant (fold change >2) changes in genes that regulate production of extra-cellular components and biofilm formation. Checkerboards demonstrated synergistic interactions between different antibiotic classes and NX, for example reducing inhibitory concentrations 2-1 to 0.25ug/mL for Gentamicin.

These results suggest potential for NX-AS-401 to be used in combination with standard of care treatments to control persistent MRSA infections

O194 CpxR Modulates Type VI Secretion System Activity in a Clinical Isolate of Serratia marcescens

Connor Bowen[1], Alex Predeus[2], Blanca Perez-Sepulveda[2], Jay Hinton[2], Sarah Coulthurst[1]

[1]*University of Dundee, Division of Molecular Microbiology, Dundee, United Kingdom*
[2]*University of Liverpool, Institute of Integrative Biology, Liverpool, United Kingdom*

Background: *Serratia marcescens* is found in a number of environments, including soil, water courses and clinical settings, where it can cause opportunistic infections. To flourish in these different niches, *S. marcescens* strains have developed a number of strategies to compete with other microorganisms. These include the production of diffusible antimicrobial molecules and the actions of contact-dependent antibacterial systems, such as the Type VI Secretion System (T6SS). The T6SS is a proteinaceous nanoweapon that can be deployed by *S. marcescens* and many other species to deliver effectors that inhibit or kill neighbouring cells.

Objectives: Much of the work on the T6SS in *S. marcescens* has been performed using the model insect pathogen, strain Db10, with a focus on the mechanism of the machinery and identification of effector proteins. Less is known about the role of the T6SS in clinical isolates of *S. marcescens*. We aim to use a multi-drug resistant clinical isolate of *S. marcescens,* SM39, to study the action and regulation of the T6SS in a pathogenic strain.

Methods: A combination of phenotypic and RNA based approaches to identify potential regulatory elements of the T6SS in SM39

Results: We show that SM39 encodes a fully functional T6SS that displays antibacterial action at environmental temperatures. We further observed that a single amino acid change in the regulator CpxR drastically changes the T6SS profile of the isolate. Our findings suggest that the regulator CpxR plays a major role in controlling the action of the T6SS in a clinical isolate of *S. marcescens.*

O196 small but mighty: a novel protein important for staphylococcus aureus virulence

Seána Duggan[1], alaa alnahari[1], eóin o'brien[2], maisem laabei[3], rachel mcloughlin[2], ruth massey[1]

[1]*university of bristol, school of cellular and molecular medicine, Bristol, United Kingdom*
[2]*trinity college dublin, biomedical sciences institute, dublin, Ireland*
[3]*university of bath, department of biology and biochemistry, bath, United Kingdom*

Background: *Staphylococcus aureus* is a human pathogen, with clinical presentations ranging from superficial skin infections to fatal bacteraemia. The emergence of antimicrobial resistance in isolates such as MRSA warrants investigation into alternative strategies for controlling *S. aureus*. Here we report a novel, small trans-membrane protein required for *S. aureus* virulence, which we have named MasA.

Objectives: This study aims to characterise the role and mechanism of MasA to *S. aureus* virulence.

Methods: A GWAS performed on a collection of MRSA isolates identified *masA* as a toxicity affecting gene. We have used multiple genetic backgrounds and infection models alongside classical microbiology, proteomics and qPCR to reveal the function of MasA.

Results: Inactivation of MasA results in a reprogramming of iron homeostasis, and higher intracellular iron levels. Furthermore, *S. aureus* requires MasA for adaptation to and recovery from iron overload. Wild type strains exposed to high levels of iron phenocopy the MasA inactivated strain's toxin production, indicating that the iron status of the *masA* mutant is involved in the reduced virulence observed. Finally, we suggest that the reduced toxicity is also mediated through repression of the major virulence regulatory system of *S. aureus*, the Agr quorum sensing system.

O197 A One Health Perspective: Faecal and Antibiotic Resistance Gene Pollution in Urban Streams and Bathing Waters

Liam Reynolds[1], Laura Sala-Comorera[1], Niamh Martin[1], Aurora Gitto[1], Tristan Nolan[1], Jayne Stephens[1], Wim Meijer[1]

[1]*University College Dublin, School of Biomolecular and Biomedical Sciences, Dublin, Ireland*

Background: Antibiotic resistance is the greatest threat to our healthcare systems. One Health recognises that the environment is shared by humans and animals and that it is a route by which bacteria harbouring antibiotic resistance genes from these sources can establish themselves in humans.

Objectives: We aimed to assess levels and sources of faecal pollution in urban streams that do not receive treated waste water and their impact on bathing waters. We also wanted to determine antibiotic resistance gene levels in these streams and bathing waters, and if these levels correlated with human and animal faecal levels.

Methods: For one year, monthly water samples were taken at Dublin city's three disignated bathing waters and two urban streams that discharge onto them. The levels of faecal indicator organisms, *E. coli* and intestinal enterococci, were ascertained and the source of faecal pollution (human, dog, gull) quantified by microbial source tracking. Also, the levels of genes conferring resistance to clinically important β-lactam, fluoroquinolone and tetracycline antibiotics were quantified by qPCR.

Results: Our results demonstrate that these urban streams receive human faecal pollution and we observed correlations between faecal indicator organism levels in the streams and the bathing waters. Furthermore, antibiotic resistance gene levels in the streams correlated with the human marker and faecal indicator organisms. No significant correlations were observed between dog and gull faecal markers and antibiotic resistance genes. These urban streams and bathing waters thus pose a public health risk as they may transmit enteric pathogens and antibiotic resistance determinants.

O198 Hyper-arid environments as a potential source of antibiotics and interactions between streptomycetes

Tiago Santos[1], Jana Schniete[1], Cristina Dorador[2], Katherine Duncan[1], RuAngelie Edrada-Ebel[1], Paul Herron[1]

[1]*University of Strathclyde, Strathclyde Institute of Pharmacy and Biomedical Sciences, Glasgow, United Kingdom*
[2]*University of Antofagasta, Departamento de Biotecnología, Antofagasta, Chile*

Background: The lack of precipitation and high solar radiation makes the Atacama Desert the ultimate arid desert on Earth and often compared to the conditions observed on Mars. As a result of adaptation to this extreme environment, microbial inhabitants might have evolved novel chemical weapons.

Objectives: Our goal was to determine the genomic relationships and interactions between closely related microorganisms inhabiting this hyper-arid environment, potentially leading to the identification of novel secondary metabolites.

Methods: Amongst many actinobacterial strains isolated from two distinct soil samples located ~30km apart, fourteen isolates with differing morphology, but possessing the same 16S rDNA sequence identical to *Streptomyces phaeoluteigriseus*, were identified. Bioactivity assays were performed to evaluate their potential to inhibit clinically relevant pathogens. Furthermore, the bioactivity between the Chilean strains was studied in order to uncover potential interactions between them. Whole-genome sequencing was also performed to determine the genomic differences between them as well as their potential to produce novel secondary metabolites.

Results: Bioactivity assays confirmed the potential of these isolates to produce secondary metabolites able to inhibit a range of clinically relevant bacteria. Interestingly, interactions between the isolates from both soils showed that isolates from one soil could inhibit isolates from the other soil but not those from the same soil suggesting a form of niche-specific adaptation with respect to antibiotic production by environmental streptomycetes.

In conclusion, fourteen isolates were characterised by their bioactivity, chemistry and whole-genome uncovering different genomic and chemical relationships due to distinctive local selective pressures in the same hyper-arid environment.

O199 The pluripotent malaria parasite: ready to go in any direction

Andrew Waters[1]

[1]*University of Glasgow, WELLCOME CENTRE FOR INTEGRATIVE PARASITOLOGY, Glasgow, United Kingdom*

Background: Malaria parasites (*Plasmodium* spp.) are an ancient scourge of human health and continue to cause significant death and morbidity in the Tropics. Transmission occurs via a female mosquito vector and requires a developmental shift to the production of transmission forms of malaria parasites (gametocytes) that are also male/female gamete precursor forms. Asexually proliferating parasites in a bloodstream infection integrates environmental cues to determine the timing, extent and quality of commitment to transmission resulting in the production of a transcription factor (AP2-G) that acts as the master regulator for gametocytogenesis.

Results: We have uncovered one aspect of the control that lies upstream of the action of AP2-G. This mechanism involves control of mRNA translation at the level of a subset of the asexual transcriptome. A specific, conserved RNA binding protein (ALBA3) is responsible for productive engagement by the ribosome of specific mRNA species that between them control the rate of progression through the blood stage asexual cycle as well as the ability to produce male or female transmission forms (gametocytes). Point mutation analysis of ALBA3 implicates protein acetylation as a key post-translational regulatory facet of commitment to sexual development. Currently we are using single cell approaches to understand if the mechanism is present in all blood stage parasites and that asexual parasite development exhibits pluripotency. It is likely that understanding of ALBA3 biology will provide significant insights into several pathological processes of both malaria and other parasite diseases and may prove amenable to targeted interruption as a control measure.

O200 Phage-encoded inhibitors of CRISPR-Cas9

Karen Maxwell[1]

[1]*University of Toronto, Biochemistry, Toronto, Canada*

Background: CRISPR-Cas systems provide bacteria with an adaptive immune system that protects them from invasion by phages, plasmids, and other foreign genetic elements. In response to this evolutionary pressure, phages evolved anti-CRISPR proteins to combat CRISPR-Cas immunity. Anti-CRISPRs are widely distributed among phage and prophage genomes and have been identified against a number of different CRISPR-Cas systems. These anti-CRISPR proteins inhibit CRISPR-Cas immunity in bacteria, as well as provide effective off-switches for CRISPR-Cas9 genome editing.

Objectives: To characterize the mechanisms by which anti-CRISPR proteins inactivate CRISPR-Cas9.

Methods: We combine *in vivo* biological assays with *in vitro* structural and biochemical analyses to identify the diverse mechanisms by which anti-CRISPR protein inhibitors block CRISPR-Cas9 activity.

Results: We have identified diverse families of anti-CRISPR proteins that inhibit the activity of CRISPR-Cas9. Several of these anti-CRISPRs show activity against distantly related Cas9 proteins. Further, diverse anti-CRISPR protein families are able to inhibit the same Cas9 functional domain via independent means. These studies provide new insight into the mechanisms by which anti-CRISPRs function and their impact on the evolution and diversification of CRISPR-Cas systems.

O201 Anti-CRISPR phages cooperate to overcome CRISPR-Cas immunity

Mariann Landsberger[1], Anne Chevallereau[1], Sylvain Gandon[2], Edze Westra[1], Stineke van Houte[1]

[1]*University of Exeter, United Kingdom*
[2]*CNRS Montpellier, France*

Background: Bacteria evolve CRISPR-Cas immunity against bacteriophage (phage) by inserting phage-derived sequences into CRISPR loci on the host genome, which can drive rapid phage extinction.

Objectives: Some phages encode anti-CRISPR (*acr*) genes, which antagonize CRISPR-Cas immune systems by binding components of its machinery, but it is unknown how these *acr* genes impact phage epidemiology.

Methods: In my talk I will present experimental data combined with epidemiological modelling predictions to explain how Acr-phages can work together to suppress CRISPR immunity of the host.

Results: In the second part of my talk I will present recent insights that can explain long-term maintenance of Acrs in a phage population.

O202 How microbes keep their CRISPR memories functional and up to date

Sebastian N. Kieper[1], Cristobal Almendros[1], Juliane Behler[3], Rebecca E. McKenzie[1], Franklin L. Nobrega[1], Anna C. Haagsma[1], Jochem Vink[1], Wolfgang R. Hess[3;4], Stan J.J. Brouns[1;2]

[1]*Delft University of Technology, Kavli Institute of Nanoscience, Department of Bionanoscience, Netherlands*
[2]*Wageningen University, Laboratory of Microbiology, Netherlands*
[3]*Genetics and Experimental Bioinformatics, Faculty of Biology, University of Freiburg, Germany*
[4]*Freiburg Institute for Advanced Studies, University of Freiburg, Germany*

The CRISPR immune system protects bacteria and archaea from invading viruses and plasmids. Immunity depends on protein diverse protein complexes that use small RNA molecules to find matching viral or plasmid DNA. I will show how viruses escape immunity by mutating target sites or glycosylating their DNA, and will highlight a mechanism called priming that updates the CRISPR memory leading to rapid co-evolution between host and phage. I will present a new mechanism catalyzed by Cas4 proteins that selects functional CRISPR memories with a PAM (protospacer adjacent motif) that is essential for efficient CRISPR targeting. We introduced the CRISPR adaptation genes *cas1*, *cas2* and *cas4* from the Type I-D CRISPR-Cas system of *Synechocystis* sp. 6803 into *Escherichia coli* and observed that *cas4* is strictly required for the selection of targets with protospacer adjacent motifs (PAMs) conferring I-D CRISPR interference in the native host. New spacers displayed variation in spacer length, which is typical for spacers found in the native I-D host and for *cas4*-containing CRISPR systems in general. We propose a model in which Cas4 assists the CRISPR adaptation complex Cas1-2 by providing DNA substrates tailored for the correct PAM. All of these insights into the biology of CRISPR gathered over the last 10 years have led to some of the most revolutionary molecular genetics tools to date, with Cas9 being the most well known example. I will review some new CRISPR tools for genome engineering approaches and molecular diagnostics.

O203 High tolerance to self-targeting in a halophilic archaeon

Aris-Edda Stachler[1], Israela Turgeman-Grott[2], Anita Marchfelder[1], Ella S. Segal[2], Thorsten Allers[3], Uri Gophna[2]

[1]*Ulm University, Germany*
[2]*Tel Aviv University, Israel*
[3]*University of Nottingham, United Kingdom*

Background: Like many systems protecting a cell against invading genetic elements, the CRISPR-Cas system can potentially cause auto-immunity. In this case, the danger lies in the acquisition of a spacer targeting the genomic DNA, resulting in an interference reaction against the genome. Self-targeting has been shown to be highly lethal for several bacteria and so far also for one archaeon: *Sulfolobus solfataricus*, a representative of the phylum Crenarchaeota.

Objectives: Here, we investigated self-targeting by the endogenous type I-B CRISPR-Cas system in the Euryarchaeon *Haloferax volcanii*.

Results: We demonstrate that expression of a self-targeting crRNA in *H. volcanii* is tolerated extremely well and does not reduce cell fitness. Even when the self-targeting crRNA is the only crRNA expressed in the cell, the observed toxic effects are modest. The surviving cells exhibit deletions in the targeted region, sequencing of the deletions indicated DNA repair via microhomology-mediated end joining.

The high tolerance of *H. volcanii* to self-targeting may result from the polyploid nature of Euryarchaeota which might provide a window of opportunity for mutations to accumulate that will render self-targeting spacers harmless.

O204 CRISPR-Cas - from biology to applications

John van der Oost[1]

[1]*Wageningen University, Laboratory of Microbiology, Netherlands*

Ten years ago major discoveries at the level of comparative genomics [1], molecular microbiology [2] and biochemistry [3] have revealed that CRISPR-Cas is a heritable adaptive immune system of bacteria and archaea, based on RNA-guided DNA interference. These key findings have initiated a revolution, including exploration the natural diversity of CRISPR-Cas classes and types [4], characterizing structure-function relations of CRISPR-associated proteins and RNA guides [5], and development of a range of applications in biotechnology and medicine [6,7]. Apart from comparing multi-subunit Cascade-like systems (class-1) and single-protein Cas9/Cas12-like systems (class-2) systems, both natural and synthetic features will be discussed that are relevant for applications [8,9].

[1] Mojica et al. (2005) J Mol Evol 60, 174-182
[2] Barrangou et al. (2007) Science 315, 1709-1712
[3] Brouns et al. (2008) Science 321, 960-964
[4] Makarova et al. (2015) Nat Rev Microbiol 13, 722-736
[5] Mohanraju et al. (2016) Science 353, aad5147/1-12
[6] Charpentier & Doudna (2013) Nature 495, 50-51
[7] Hsu et al. (2014) Cell 157, 1262-1278
[8] Zetsche et al. (2017) Nat Biotechnol 35, 31-34
[9] Wu et al. (2018) Nat Chem Biol. 14, 642-651

O205 Rapid strain development of Corynebacterium glutamicum for heterologous production of squalene using multiplex CRISPR interference and high-throughput fermentation

Jaehyun Park[1], Han Min Woo[1]

[1]*Sungkyunkwan University, Food Science and Biotechnology, Suwon, Korea, Rep. of South*

Background: Resources of natural squalene, a valuable triterpene used in the food, personal care, and medical industries, have been limited because of low-yielding bioprocesses from plants and social-environmental changes related to harvesting shark oil. Thus, the sustainable production of squalene has driven the development of microbial cell factories (MCFs).

Objectives: Efficient strain development of *Corynebacterium glutamicum* has been aimed to produce heterologous squalene from glucose.

Methods: Combinatorial metabolic engineering strategies for precursor rebalancing, redox balancing, and blocking the competing pathway for the isopentenyl diphosphate availabilities were applied by repressing the target genes using Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) interference.

Results: The best engineered strain using high throughput fermentation produced squalene from glucose at 5.4 ± 0.3 mg/g DCW and 105.3 ± 3.0 mg/L, which was a 5.2-fold increase over the parental strain. In addition, flask cultivation of *C. glutamicum* overexpressing the *dxs* and *idi* genes with the truncated squalene synthase gene and repressing the *idsA* gene resulted in production of squalene at 5.8 ± 0.4 mg/g DCW and 82.8 ± 6.2 mg/L, which was a 3.4-fold increase compared with that of the parental strain. This report demonstrates that blocking the competing pathway using the CRISPRi in *C. glutamicum* was effective in enhancing the squalene production.

O206 Temperature, by Controlling Growth Rate, Regulates CRISPR-Cas Activity in Pseudomonas aeruginosa

Nina Molin Høyland-Kroghsbo[1], Katrina Arcelia Muñoz[2], Bonnie L. Bassler[2]

[1]*University of Copenhagen, Department of Veterinary and Animal Sciences, Frederiksberg, Denmark*
[2]*Princeton University, Department of Molecular Biology, Princeton, United States*

Background: Clustered regularly interspaced short palindromic repeat (CRISPR)-associated (CRISPR-Cas) systems are adaptive defense mechanisms that protect bacteria and archaea from invading genetic elements, such as plasmids and phages. CRISPR RNAs guide Cas protein complexes to cleave complementary foreign nucleic acids, a process known as interference. The resulting short fragments of cleaved DNA can be incorporated into the genomic CRISPR array. This adaptation step expands the genetic memory of prior infecting elements stored in CRISPR.

Objectives: We hypothesized that slowing bacterial growth could buy a bacterium more time for its CRISPR-Cas machinery to successfully adapt to and eliminate a foreign genetic element prior to being parasitized or killed.
Methods: We used mutagenesis and molecular analyses to test the effect of low temperature and slow growth on adaptation and interference against a plasmid, in the type I-F CRISPR-Cas system of *P. aeruginosa* PA14.

Results: We have discovered that temperature regulates CRISPR-Cas interference and adaptation, i.e., target cleavage and acquisition of new immunity spacers, respectively, in *P. aeruginosa* PA14. Specifically, at low temperature, CRISPR-Cas complex levels increase and growth rate decreases, each of which promotes increased adaptation. This presumably provides additional time prior to cell division for CRISPR-Cas to patrol the cell and successfully eliminate and/or acquire immunity to foreign DNA.

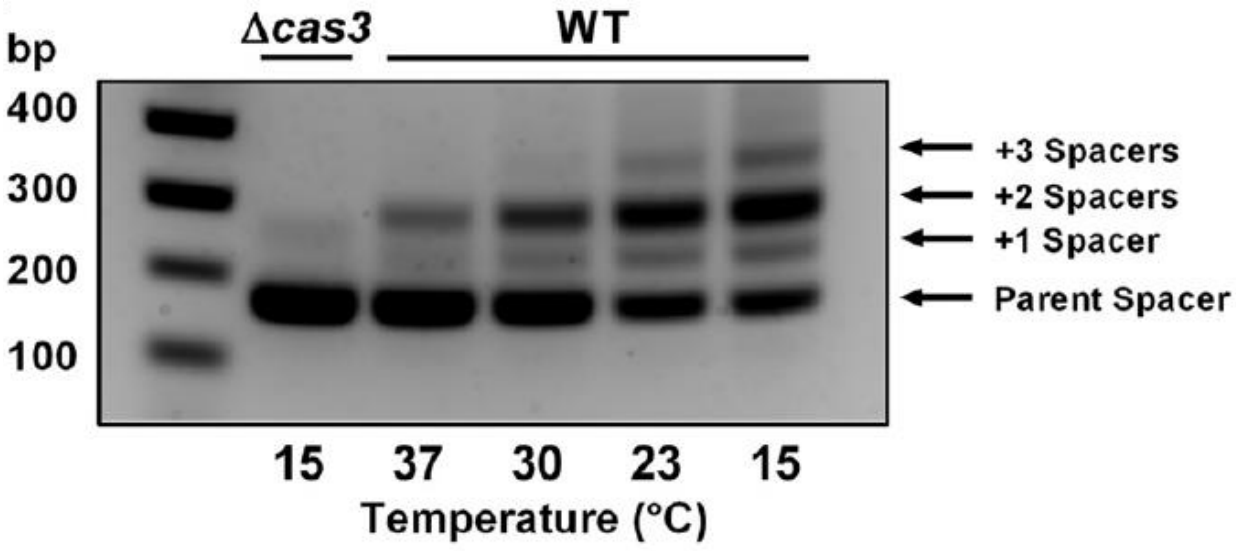


Fig. 1. CRISPR adaptation detected by PCR and gel electrophoresis of the CRISPR2 locus of PA14 and the interference deficient Δ*cas3* mutant. Each adaptation event is exhibited by a 60 bp increase.

O207 Characterizing an antiCRISPR-based on/off switch for bacterial genome engineering

Ioannis Mougiakos[1], Despoina Trasanidou[1], Prarthana Mohanraju[1], Richard van Kranenburg[2], John van der Oost[1]

[1]*Wageningen University & Research, Wageningen, Netherlands*
[2]*Corbion, Gorinchem, Netherlands*

Background: CRISPR-Cas9 technologies have enabled unprecedented efficient genome editing and transcriptional regulation in prokaryotes, allowing for their accelerated exploration and exploitation. However, tight control of Cas9 expression requires either multi-step approaches, or synthetic fusions of Cas9 and specific sensor proteins that respond to external stimuli.

Objectives: In this study we aimed to harness the function of a small anti-CRISPR protein from *Neisseria meningitidis* ($AcrIIC1_{Nme}$) as natural and direct "on/off-switch" of two thermo-tolerant Cas9 orthologues, from *Geobacillus thermodenitrificans* T12 (ThermoCas9) and *Geobacillus stearothermophilus* (GeoCas9).

Methods: We employed episomal based expression of the $AcrIIC1_{Nme}$ in *Escherichia coli* and combined it with episomal based expression of either ThermoCas9 or GeoCas9, using single- and double-vector approaches.

Results: We demonstrate that both ThermoCas9 and GeoCas9 are *in vivo* active at 37°C and can be used for introducing dsDNA breaks in *E. coli*, in a tunable and spacer-dependent manner. In addition, we show that $AcrIIC1_{Nme}$ traps *in vivo* these Cas9 endonucleases in a DNA-bound, catalytically inactive state, robustly inhibiting targeting and resulting in a transcriptional silencing that is comparable to their catalytically "dead" variants (Thermo-dCas9 and Geo-dCas9). Moreover, we describe a single-vector, tightly controllable and highly efficient Cas9/$AcrIIC1_{Nme}$-based tool for silencing in bacteria. Altogether, an anti-CRISPR protein has been used to control Cas9-based genome editing and transcriptional regulation.

O208 Sequence-specific re-sensitization of drug resistant bacteria using CRISPR-Cas9: first in vivo outcomes

Thaysa Tagliaferri[1;2], Liza Felicori[3], Tiago Mendes[4], Simone Santos[1], Hans-Peter Horz[2]

[1]*Federal University of Minas Gerais, Microbiology, Belo Horizonte, Brazil*
[2]*RWTH Aachen University, Aachen, Germany*
[3]*Federal University of Minas Gerais, Brazil*
[4]*Federal University of Viçosa, Brazil*

Background: Considering that the emergence and spreading of antimicrobial resistance genes is much faster than the discovery of new antimicrobials, alternative strategies, such as reversing resistance using CRISPR-Cas9 may have a significant impact for future clinical applications.

Objectives: To reverse resistance encoded by the bla_{TEM} gene and to evaluate the potential of antibiotics to rescue larvae from infection with *E. coli* treated or untreated by CRISPR-Cas9.

Methods: First, a gRNA was designed to target a highly conserved region of the bla_{TEM} gene, and inserted into a CRISPR-Cas9 vector. After transformation into *E. coli*, the functionality of the CRISPR-Cas9 system was verified by qPCR of the RNA transcripts. The drug-resistance phenotype was assessed by bacterial growth behavior in the presence or absence of beta-lactam antibiotics. Larvae of *Galleria mellonella* were infected with defined amounts of drug-resistant or re-sensitized *E. coli* strains in order to evaluate the antibiotic rescue capacity upon phenotype reversal.

Results: Successful expression of the Cas9 enzyme in the transformed *E. coli* cells could be demonstrated in all experiments by qPCR. Growth curves as well as disk diffusion tests confirmed full resistance reversal in *E. coli*. After 72h of infection with the re-sensitized *E. coli* 70% of the larvae could be rescued by administration of ceftriaxone as opposed to 38% of larvae infected by the *E. coli* wild type. Thus, this study shows that the sequence specific alteration of bacteria efficiently reverses drug resistance and that the treatment with traditional antibiotics is possible in *in vivo* infection models.

O209 Who Moved My Cheese and its relevance to Microbiology Teaching

Laura Bowater[1]

[1]*The University of East Anglia, Norwich Medical School, Norwich, United Kingdom*

Background: Future microbiology graduates are destined to enter a future, career environment, which has changed substantially over the last two decades. In contrast, it could be argued that current approaches for teaching microbiology and encouraging the development of learning skills has not kept pace with these changes. Who Moved My Cheese is an allegory for how change can be dealt with in the work-place.

Objectives: This session will allow delegates to question and discuss whether;

- We need to re-examine and change both the methods and the subject matter that we currently provide to our microbiology students?
- The opportunities we provide for undertaking research are fit for purpose in a modern microbiology curriculum?

Methods: A brief *Introduction to Who Moved My Cheese*, will be provided by the speaker. This session will provide an Introduction to the concept that there is both, a need for and a method to cope with the changes in the learning environments encountered by researchers and educators working in a modern, higher education sector. This introduction will be followed by a short panel discussion to include speakers from *The Research Teaching Nexus: Exploring its role in Microbiology* and members of the audience. Topics covered may include the need for change and the capacity for individuals to thrive in a rapidly changing higher educational landscape.

Results: This session will encourage participants to consider whether there are any changes that educators of future microbiology workforces can adopt to improve their teaching practice.

O210 The future of research-led teaching in (micro)biology: not different, just better

Joseph Gray[1]

[1]*University of Glasgow, School of Life Sciences, Glasgow, United Kingdom*

Background: Two core activities for academic (micro)biologists are 1) doing original scientific research and 2) educating university students. The link between good education and active research is often assumed but is rarely scrutinized, and thus unlikely to be optimized. Few or us are expert in education: for our teaching, we default to doing what was done to us by an earlier generation of scientists. Hence change has been slow in (micro)biology education over the last 25 years. But the world around us has changed hugely: how students learn is understood much better; the number of graduates has increased hugely; the job market has expanded and diversified. Our graduates are not all clones of us, nor do they wish to be.

Objectives: To consider the question: What can we do better?

Methods: In this short lecture, I will start with the state of practical/project work in undergraduate programmes as of 2014 (in work done with Kevin Coward at Oxford). I will summarize what a university degree in microbiology should already be doing for our students (according to QAA benchmark statements, 2015) and will highlight where we might be falling short.

Results: I will argue that we need evolution, not revolution: to do things better by creatively putting *more* of our scientific research expertise at the very core of what our students actively do, and of how we assess them.

O211 The Perceptions of Research and Research-led Teaching

Laura Bowater[1], Elena Nardi[2], Kay Yeoman[3]

[1]*University of East Anglia, Norwich Medical School, United Kingdom*
[2]*University of East Anglia, School of Education, United Kingdom*
[3]*University of East Anglia, School of Biological Sciences, United Kingdom*

Background: Research-led teaching is a concept which features in the mission statements of higher education institutes. It is an approach which is used by research intensive universities to show the link which exists between research and good teaching. But what does term research actually mean to a university student and how does this effect their understanding of what research led-teaching is? Undergraduate students are heavily influenced by their school experiences, so it is important to look at how their ideas around research have been shaped by their interactions with research in secondary school.

Objectives: To understand what the term research means to pupils in secondary school and how this has shaped understanding of research and the research process in higher education.

Methods: A questionnaire and structured interviews were conducted. The 25-question survey was split into three themes: 1, who does research? 2 ,what is the value of research? 3, how do we do research? The English examination boards and the national curriculum were examined as to how the word 'research' is used in context. This study was extended to undergraduate and postgraduate students to determine the point at which their understanding of research changes.

Results: Secondary school pupils were confident that they could do research, but they considered research to be fact-finding, and not necessarily a process towards something new. This study was then extended to both undergraduate and postgraduate students at university to try and ascertain the point at which their understanding of research changed.

O212 The Research-Teaching Nexus: Quo Vadis

Beatrix Fahnert[1]

[1]*University of Lincoln, School of Life Sciences, Lincoln, United Kingdom*

A teacher's practice will usually be situated somewhere within the research-teaching nexus, even if they are not familiar with the nexus concept. Practice naturally varies, e.g. depending on the subject, but even if we just focus on microbiology, practice has been changing in context of changes in Higher Education and respective institutional strategies. Given the variations in interpretation and implementation, it is worth looking into those developments to ensure the outcomes are as intended.

Institutions often use the term research-led teaching to convey that students learn in and have access to an environment with excellent research output. However, the term research-informed would be more appropriate. With an emphasis on content, we fairly routinely find research-led teaching (students are exposed to research findings) e.g. in lectures. The traditional capstone research project would be an example of research-based teaching, where students actively enquire and the emphasis is on the process.

With the HE sector having continuously seen changes, such as increasing student numbers while only some students seek a research career, we are challenged to keep the curriculum relevant and sustainable. Hence, institutions strategically focus on certain aspects within the nexus. As research-based learning activities decrease, extracurricular research projects become sought-after opportunities, which stretch research-interested students and sometimes also offer interdisciplinary and intercultural experiences.

This session will explore strengths and weaknesses of trends and emerging patterns of research-informed teaching in context of student learning and experience, use of staff expertise and outcomes for the institution.: Objectives: Methods: Results:

O213 Antimicrobial resistance a true One health issue

Mark Fielder[1], Simon W. Gould[1], Fredericka Mitchell[1], Gary Forster-Wilkins[1], Lauren Mulcahy-Ryan[1], Adam LeGresley[1], Tim Potter[2], Matt Dobbs[2]

[1]*Kingston University, Faculty of Life Science, Pharmacy and Chemistry, United Kingdom*
[2]*Westpoint Farm Vets, United Kingdom*

Background: Whilst the spectre of antimicrobial resistance has been ever present, its importance in recent years has become more apparent. This has lead to questions as to the sources or resistance and of course how it might be monitored and controlled. The O'Neill reports emenating from the UK have indicated a number of aspects that should be focussed upon protection and fostering the best use of antimicrobials – these include the development of rapid diagnostic tools for use at the point of care. Tools such as these can potentially have value across one health and help in the targeted treatment of infections where identified.

Objectives: Demonstrate the potential of novel tests to effectively function in different settings across one health and so inform the diagnostic and or prescribing process.

Methods: This presentation discusses the development of two candidate rapid diagnistic tests using novel substrate analogues for the detection of pathogen specific enzymes following the release of a fluorophore. The second test employs the combined use of molecular biology and serology to detect not only pathogen specific genes but also the organism itself.

Results and Discussion: Both tests described are potentially capable of detecting their respective targets direct from the p[atient without the need for further culture. There are candidate diagnostics that can operate in a one health context capable of providing relevant data that can help to inform treatment choices. These tests and their develoment and application will be discussed.

O214 The Global Research on AntiMicrobial resistance (GRAM) project: determining the global burden of antimicrobial resistance

Catrin Moore[1]

[1]*University of Oxford, Nuffield Department of Medicine, Oxford, United Kingdom*

Background: The Global Research on AntiMicrobial resistance (GRAM) project is a partnership between the University of Oxford and the Institute of Health Metrics and Evaluation (IHME) in Seattle. The project aims to provide robust, comprehensive and timely evidence of the global burden of antimicrobial resistance (AMR) in 195 countries and territories.

Objectives: The work has initially focused on 17 bacteria-antibacterial drug ("bug-drug") combinations, but this will be expanded over the coming months, we aim to work collaboratively with researchers and public health officials to: i) Consolidate, review and analyse all available data and scientific information on AMR worldwide (1990 onwards); ii) Produce granular geospatial maps of AMR burden as detailed as the data will allow; and iii) Promote the widespread dissemination of results using tools and interactive data visualizations.

Methods: The methods will consist of i) Quantification of the burden of disease, (number of deaths, disease incidence and prevalence, by age, sex, time and location, for each cause/clinical syndrome with pathogens) based on the ICD underlying causes, to calculate the fraction of DALYs attributable to AMR ii) Determine the prevalence of antibiotic drug resistance by pathogen by underlying cause of death, sample source, hospital/community origin, etc. and iii) Determine the excess risk of death or adverse outcome by pathogen and drug resistance compared to antimicrobial sensitive health outcomes.

Results: This work will generate comparable AMR burden estimates across all 195 countries together with geospatial maps with the granularity dependent upon the data available.

O215 Antimicrobial resistance in pets, whats the threat?

Nicola Williams[1]

[1]*University of Liverpool, Epidemiology and Population Health, Neston, United Kingdom*

Background: Antimicrobials are important for managing infectious disease in dogs and people have close and frequent contact with dogs, therefore they may represent a link in the development and transfer of antimicrobial resistance (AMR). Of concern is the emergence of extended-spectrum producing B-lactamases (ESBLs) and AmpC b-lactamases conferring resistance to the 3rd generation cephalosporins (3GCR).

Objectives: To determine the prevalence, risk factors and molecular epidemiology of AMR and 3CGR *Escherichia coli* in dogs in the community, veterinary hospitals, boarding and rescue kennels and in people who have occupational exposure to dogs.

Methods: Faecal samples were collected from dogs and people and *E. coli* cultured for the detection of AMR and ESBL-producing *E. coli*. All isolates were subject to standard antimicrobial susceptibility and PCR assays used to detect keys genes and whole genome sequencing performed on key isolates.

Results: Low prevalences of resistant *E. coli* were observed in the vet visiting dog population to 3CGRs, fluoroquinolones and potentiated amoxicillin, however the feeding of raw meat diets was a significant risk factor for 3CGR *E. coli* carriage. Higher prevalences of 3CGR *E. coli* were found in hospitalised dogs. The prevalence of ESBL-producing *E. coli* in people working in vet practices, dog rescue and boarding kennels was however similar to that reported in the community in other countries. Furthermore, whilst there was evidence of particular 3CGR *E. coli* strains or plasmids, shared in dogs or people in the same premises, this occurred more rarely in both dogs and people.

O216 One Health Approaches to Predicting and Preventing Emerging Viruses

Peter Daszak[1], Mark Fielder[2], Aleksei Chmura[1;2], Hongying Li[1]

[1]*EcoHealth Alliance, United States*
[2] *Kingston University, United Kingdom*

Most high-impact emerging diseases are caused by zoonotic viruses. They emerge through a complex interaction of socio-economic and environmental drivers acting on contact networks among wildlife, livestock and people. Our organization uses field studies and ecological analyses to trace back the origins of specific diseases, estimate viral diversity in wildlife reservoirs, and identify the geographical regions and global changes most likely to produce pandemics. Using examples from SARS-CoV, MERS-CoV, SADS-CoV and others, I will show how a One Health approach can provide answers to critical questions for emerging viruses, including:

1. Where will the next emerging virus originate?
2. What are the key causes of disease emergence?
3. Which reservoir species will the next EID likely emerge from?
4. How many unknown viruses do these species harbor, and how many can infect us?
5. Can we predict and prevent emergence, and is the expense worth it?

Reducing the risk of new emerging diseases is a key scientific challenge that will require global cooperation because novel viruses mainly originate in tropical regions, but have the highest economic impact on richer, rapidly developing countries in the North. Despite the exponential increase in EID threats, our work shows that cooperative strategies to combat these threats will provide significant return-on-investment as well as public health benefits.

O217 Synthetic microbiology: Design and development of industrial cell factories

Christoph Wittmann[1]

[1]*Saarland University, Institute of Systems Biotechnology, Saarbrücken, Germany*

Background: Superior microorganisms that produce chemicals, materials, fuels, and health care products from renewables are major drivers of the developing bio-based economy. They are the key to enable sustainable production from green resources rather than from fossil fuels.

Objectives: Such industrial cell factories have to operate as empowered synthetic machines, very different from the typically low producing wild tpyes. They should be highly efficient in terms of catalytic power and enable optimum performance at high titer, yield, productivity, and selectivity. Furthermore, they have to tolerate harsh production conditions and manage to convert second and third generation feedstocks, often crude and partly toxic mixtures of substrates. This requires novel concepts of synthetic microbiology for strain design and development.

Methods: Synthetic microbiology integrates systems biology and systems biotechnology with metabolic engineering and synthetic biology to enable a global analysis, design, and engineering of the underlying complex metabolic and regulatory networks and bioprocesses at an efficiency and versatility otherwise not accessible. In this sense, it is changing the way to design and optimize microbial cell factories.

Results: Meanwhile, model-based metabolic design and engineering can breed synthetic strains, which reach the high performance of classical producers derived over the past decades. This lecture will highlight superior efficient cell factories and bioprocesses for bio-production of industrial chemicals and materials to illustrate these concepts by. In addition, it will present novel approaches that extend the raw material basis to third generation renewables towards a sustainable green chemistry.

O218 RecET recombineering system for markerless integration of heterologous biosynthetic gene clusters to Pseudomonas putida chromosome

Kyeong Rok Choi[1], Jae Sung Cho[1], In Jin Cho[1], Dahyeon Park[1], Sang Yup Lee[1]

[1]Korea Advanced Institute of Science and Technology, Department of Chemical and Biomolecular Engineering, Daejeon, Korea, Rep. of South

Background: *Pseudomonas putida* has been considered as a promising workhorse of metabolic engineering, particularly for production of valuable natural products. However, integration of heterologous genes into the *P. putida* chromosome, which is essential to generate stable industrial strains producing heterologous bioproducts, rely on time-consuming homologous recombination and transposon-mediated random insertion techniques. More efficient method for progammed integration of heterologous genes to *P. putida* chromosome is required.

Objectives: A RecET recombineering system for markerless integration of heterologous genes into the *P. putida* chromosome was developed.

Methods: Various genetic loci on the *P. putida* chromosome were knocked out to evaluate the RecET reombineering system. Cre/*lox* system and plasmid curing systems completed the markerless recombineering system. In addition, donor plasmid system was developed for efficient preparation of donor DNAs and their delivery to *P. putida* for markerless integration of heterologous genes.

Results: The efficiency and capacity of the recombineering system were first demonstrated by knocking out various chromosomal loci spanning 0.6 – 101.7 kb. In addition, the RecET recombineering system allowed successful markerless integration of four different biosynthetic gene clusters of protein, polyketide, isoprenoid, and amino acid derivative. This markerless recombineering system for efficient gene knockout and integration will expedite metabolic engineering of *P. putida* for heterologous production of valuable natural products. [This work was supported by Novo Nordisk Foundation grant NNF16OC0021746 and Technology Development Program to Solve Climate Changes on Systems Metabolic Engineering for Biorefineries (NRF-2012M1A2A2026556 and NRF-2012M1A2A2026557) from the Ministry of Science and ICT through the National Research Foundation (NRF) of Korea.]

O219 Genetic Modules to Use Enforced ATP-Wasting as a Tool for Metabolic Engineering

Simon Boecker[1], Ahmed Zahoor[1], Steffen Klamt[1]

[1]*Max Planck Institute for Dynamics of Complex Technical Systems, Analysis and Redesign of Biological Networks, Magdeburg, Germany*

Background: ATP-wasting has been proposed as a tool to improve the productivity and yield of desired metabolites by cell factories [1]. The concept consists of two steps: First, a knock-out strategy is established to make the (by)production of a target metabolite mandatory for net ATP synthesis. This is followed by enforced ATP wasting (e.g. by overexpression of ATPases or futile cycles), thereby forcing the cells to increase ATP synthesis to keep up with the cellular maintenance demand and simultaneously leading to higher specific productivity and yield.

Objectives: Providing a proof-of-principle for the use of enforced ATP wasting to improve production performance of microbial strains for synthesis of ATP-coupled products.

Methods: Inducible expression of the cytosolic F_1-subunit of the *E. coli* ATPase (catalyzing the hydrolysis of ATP to ADP and P_i) in wild type *E. coli* and *S. cerevisiae* strains and analyzing its influence on the fermentative product formation under anaerobic growth conditions.

Results: Overexpression of the ATPase resulted in approx. 10% increase in titer, yield and specific productivities of *E. coli* fermentation products. However, as growth rates decreased, the volumetric productivity dropped compared to the wild type strain. This issue was addressed by applying a two-stage-process, where growth and production phases are decoupled [2]. With this approach, the volumetric productivity could be doubled during the production phase, making this concept interesting for industrial applications.

[1] Hädicke *et al*., *Biochem Soc Trans* **2015**, 43, 1140-1145

[2] Klamt *et al*., *Biotechnol J* **2018**, 13, 1700539

O220 Wastebot: An Automatic Platform for the Optimisation of Engineered Biological Systems

Lucile Chatellard[1], Alastair P. Thurlbeck[2], Kohei Motoya[3], Soichiro Tsuda[3], Geoffrey J.T. Cooper[4], Stephanie Connelly[1], Leroy Cronin[4], William Sloan[1]

[1]University of Glasgow, School of Engineering, Division of Infrastructure and Environment, Glasgow, United Kingdom
[2]Oregon State University, Energy Systems Group, School of Electrical Engineering and Computer Science, Corvallis, United States
[3]Osaka University, Graduate School of Frontier Biosciences, Suita, Osaka prefecture, Japan
[4]University of Glasgow, WestCHEM, School of Chemistry, Glasgow, United Kingdom

Background: Optimisation of bioprocesses is highly challenging due to the large number of variables to be tested. Thus, bioprocess development is time-consuming and cost-intensive. As demonstrated in pharmaceutical and agri-food fields, automation of microscale bioprocess optimisation can significantly reduce the time and cost of bioprocess development. Here we propose automated optimisation can be adapted to environmental bioprocesses.

Objectives: Building on precedent in digital chemistry, we have developed a robotic platform targeting optimisation engineered biological systems by automated optimisation of the underlying microbiology.

Methods: Coupling open-source software with 3D-printing of bespoke components and a simple webcam to monitor bacterial growth, we have developed a low-cost robotics platform for microbial optimisation in liquid cultures. Performance of the platform was verified against that of a commercial microplate reader (Tecan 2000) in determination of a Monod curve for E. coli MG1655 using a simple growth media. Automatic optimisation of culture conditions for the rapid growth of bacteria is currently in development using simple algorithms.

Results: We demonstrate that using a simple camera to monitor bacterial growth, we observe similar growth parameters as when using traditional spectrophotometric techniques. Further, we determine that the robotics platform and software can be used to estimate Monod parameters with similar accuracy to the plate reader and with minimal user. From this preliminary experiment, we used the Golden Search algorithm to optimise one culture condition: the pH. Future works will extend our algorithmic approach to the automated optimisation of multiple culture parameters and to bioprocesses using mixed microbial communities.

O221 Engineering Pseudomonas putida for the production of high value aromatics from plant waste material

James Williamson[1], Tim Bugg[1]

[1]*University of Warwick, Chemistry, Coventry, United Kingdom*

Background: Grasses such as wheat and sugar cane produce ferulic acid to synthesise ether ester linkages between lignin and hemicellulose. Aromatic degrading bacteria, such as *Pseudomonas putida*, have pathways that utilise ferulic acid via the beta ketotadipate pathway, and therefore could be useful organisms for releasing aromatics from agricultural waste.

Objectives: This work seeks ways to valorise waste plant material from British agriculture and Brazilian biofuel production, such as wheat straw and bagasse, by producing high value aromatic compounds.

We aim to produce strains of bacteria that are able to firstly release ferulic acid from dried lignin sources, and then develop pathways to higher value products.

Methods: Initial work has revolved around identifying and disrupting specific aromatic degredation pathways in *P. Putida,* so as to produce a strain capable of accumulating ferulic acid when grown on wheat straw.

The next stages are focusing on developing biosynthetic pathways for the production of coniferyl alcohol and chalcones.

Work has also been done to produce a ferulic acid biosensor strain of *P. putida* which can help us better understand the degredation of ferulic acid, as well as provide a potential ferulic acid inducible promoter system to provide a feedback system for the biosynthetic pathways.

Results: Currently we have a strain of *P. Putida* which can accumulate ~80 mg/l of ferulic acid fter being grown on 2% wheat straw. Pathways are being developed for the production of coniferyl alcohol and naringenin and integrated into the genome under control of the ferulic acid biosensor.

O222 Engineering of the citrate exporter protein enables high citric acid production in Aspergillus niger

Matthias Steiger[1], Alice Rassinger[1], Diethard Mattanovich[1], Michael Sauer[1]

[1]*Muthgasse 18, Department of Biotechnology, Wien, Austria*

Background: The filamentous fungus *Aspergillus niger* is capable of producing citric acid and is used for large scale fermentations for more than a century. However, the information on the genetic identity of the *A. niger* citrate transport system, particularly the components involved in the export system over the plasma membrane are missing.

Objectives: The objective of this work is to identify the genes involved in citrate transport system of *A. niger*. Identified target genes shall be further characterized and used to engineer the citric acid secretion capability of *A. niger*.

Methods: Candidate genes are characterized by a gene deletion strategy screening for strains with reduced citric acid secretion. An identified target gene was characterized by heterologous expression in *Saccharomyces cerevisiae* and by overexpression in *A. niger*.

Results: A transport gene (*cexA*) was identified and the encoding protein, CexA, belongs to the major facilitator superfamily subclass DHA1 including drug-H^+ antiporter. Disruption of *cexA* completely abolishes citric acid secretion showing that this protein is the main citric acid transporter in *A. niger*. The gene can be heterologously expressed in *S. cerevisiae*, which leads to the secretion of citric acid during the growth on glucose. Overexpression of *cexA* leads to a significant increase in secreted citric acid in *A. niger*. With an inducible expression system strains yield up to 109 g/L citric acid, which is 5 times higher compared to the parental strain. These results demonstrate the importance of the cellular transport system for an efficient production of metabolites.

O223 Substantial improvement of toyocamycin production in Streptomyces diastatochromogenes by cumulative drug-resistance mutations

Xuping Shentu[1], Zhenyan Cao[1], Yin Xiao[1], Gu Tang[1], Kozo Ochi[2], Xiaoping Yu[1]

[1]Zhejiang Provincial Key Laboratory of Biometrology and Inspection & Quarantine, College of Life Sciences, China Jiliang University, Hangzhou, China
[2]Department of Life Science, Hiroshima Institute of Technology, Hiroshima, Japan

Background: Toyocamycin is a member of the nucleoside antibiotic family and has been recognized as a promising fungicide for the control of plant diseases. However, low productivity of toyocamycin remains an important bottleneck in its industrial production.

Objectives: Dramatic improvements of strains for overproduction of toyocamycin are of great interest in applied microbiology research.

Methods: Mutations were selected from multiple drug (streptomycin, gentamicin, paromomycin) resistance plates by ribosome engineering.

Results: In this study, we sequentially selected for mutations for multiple drug resistance to promote the overproduction of toyocamycin. The triple mutant strain, SD3145 (*str str par*), was obtained through sequential screenings. This strain showed an enhanced capacity to produce toyocamycin (1500 mg/L), 24-fold higher than the wild type in GYM liquid medium. This dramatic overproduction was attributed at least partially to the acquisition of an *rsmG* mutation and increased gene expression of *toyA*, which encodes a LuxR-family transcriptional regulator for toyocamycin biosynthesis. The expression of *toyF* and *toyG*, probably directly involved in toyocamycin biosynthesis, was also enhanced, contributing to toyocamycin overproduction. By addition of a small amount of scandium ($ScCl_3 \cdot 6H_2O$), the mutant strain, SD3145, produced more toyocamycin (2664 mg/L) in TPM medium, which was the highest toyocamycin level produced in shake-flask fermentation by a streptomycete so far. We demonstrated that introduction of combined drug resistance mutations into *S. diastatochromogenes* 1628 resulted in an obvious increase in the toyocamycin production. The triple mutant strain, SD3145, generated in our study could be useful for improvement of industrial production of toyocamycin.

O224 Engineering the expression of chromosomal cytosine deaminase in an E. coli strain with targeted adhesion to tumor cells for increased specificity of enzyme-prodrug cancer therapies

Elena M. Seco [1], Carmen Mañas[1], Luis Angel Fernandez[1]

[1]*Centro Nacional de Biotecnología (CSIC), Department of Microbial Biotechnology, Madrid, Spain*

Background: Cytosine deaminase (CD) converts the non-toxic 5-fluorocytosine (5-FC) to the highly toxic 5-fluorouracil (5-FU), leading to cell death by misincorporation of 5-FU during RNA and DNA synthesis. 5-FU is a chemotherapeutic drug widely used in the treatment of different cancers despites its toxicity toward non-tumor cells.

Objectives: Enhance the specific killing of tumor cells by engineering the inducible expression of the chromosomal CD in an *E. coli* strain with targeted adhesion to tumor cells.

Methods: A genetic cassette encoding tetR-PtetA was inserted upstream of the endogenous *codA* (CD) in the genome of an *E. coli* strain that attach specifically to human tumor cells expressing the epidermal growth factor receptor (EGFR). The CD activity of the modified strain was determined and the cytotoxicity of 5-FU product was assayed in vitro using bladder and colon tumor cell lines.

Results: High level expression of CD was detected in the presence of the inducer anhydrotetracycline (aTc). The enzymatic activity of CD in the induced bacterial extracts revealed an efficient conversion of 5-FC to 5-FU. Colon and bladder tumor cells lines expressing EGFR were sensitive to low concentrations of 5-FU, which did not affect the growth of the engineered *E. coli* strain. Conversion level of 5-FC to 5-FU by the engineered *E. coli* was sufficient to inhibit growth and cause cell death of bladder and colon tumor cells. Hence, the engineered bacterial strain has the potential to be used in CD/5-FC prodrug therapy against different human tumors and will be tested in animal models.

O225 Saccharomyces cerevisiae and Lactobacillus plantarum cooperation in a synthetic wine environment

Sandra Christine du Toit[1], Debra Rossouw[1], Maret du Toit[1], Florian F. Bauer[1]

[1]*Stellenbosch University, Viticulture and Oenology, Stellenbosch, South Africa*

Background: *Saccharomyces cerevisiae* and *Lactobacillus plantarum* are phylogenetically distant species, but co-exist in environments such as grape juice, where they are responsible for alcoholic and malolactic fermentation (AF and MLF) respectively. Understanding how these species interact with each other and their environment is important to better manage successful completion of AF and MLF. However, the complexity of the wine matrix makes it difficult to study these interactions in a natural environment and synthetic ecological systems can therefore be used to overcome these difficulties.

Objectives: The study was designed to establish a co-dependent, mutualistic relationship between *S. cerevisiae* and *Lb. plantarum* in order to gain insights into the cooperation between species and to determine the effect of pH and temperature on the relationship.

Methods: The interaction, centred on the reciprocal exchange of amino acids, was established between *S. cerevisiae* BY4742Δ*thi4* (lysine auxotroph) and *Lb. plantarum* IWBT B038 (isoleucine, alanine, valine, and methionine auxotroph) by omitting different combinations of amino acids from the chemically defined synthetic grape juice-like media.

Results: An obligatory co-dependency was established between the *Lb. plantarum* and *S. cerevisiae* strains. Lower temperature and lower pH had a negative effect on the growth and malic acid consumption of *Lb. plantarum* which could be alleviated in the co-cultures with yeast, suggesting mutual beneficial impacts of co-cultivation. The study provides insights into the interaction between these species in a synthetic wine environment and provides a basis for future work to create optimised yeast-bacteria pairings through directed evolution for potential applications in winemaking.

O226 The human microbiome: a disruptive idea that provides insight into human biology

Julian Marchesi[1]

[1]*Imperial College & Cardiff University*

Background: The concept of super organism biology has been a powerful driving force in trying to integrate different microbiome's into human biology. The whole area of the human microbiome is also a disruptive idea which is dramatically altering the way in which we consider the way in which non-communicable diseases are increasing in the human population. Moreover, we are starting to understand how the human microbiome may also play a role in how we treat these diseases. In this presentation

Objective: To explore these concepts and how the new field of faecal microbiota transfer (FMT) is starting to allow us to understand different aspects of how the gut microbiota controls diseases. I will specifically focus on how we can use the process of FMT to develop new therapeutic avenues potential future directions that this may take.

Methods: Using poly-omic approaches we have dissected the different aspects of FMT in the context of *Clostridioides difficile* associated infections (CDAI).

Results: Poly-omic approaches have identified that 2 components are all that is needed to mimic full FMT treatment of CDAI and that the presence of viable bacteria is not an essential criteria for successful treatment. This is an exemplar scenario of how we can use FMT to explore host –microbe interactions.

O227 Diet and Microbiota in Health of the elderly

Paul O'Toole[1]

[1]*University College Cork, APC Microbiome Ireland & School of Microbiology, Cork, Ireland*

Background: Distinct microbiota composition groups in older people corresponding to habitual diet. Correlations in the data between diet, microbiota and health status suggested a causative axis

Objectives: To investigate diet-microbiome interaction in murine models and by prebiotic supplementation in humans

Methods: We colonized germ-free or conventional mice with high-diversity gut microbiota or low diversity microbiota from healthy or frail subjects, respectively. Recipient mice retained microbiota similar to that of the donor when maintained on a synthetic diet modelled that of the donor type. We supplemented the human diet with 5 prebiotic ingredients, up to 21 g per day for 6 months, or placebo. 100 subjects were enrolled from three strata; elderly community-dwelling subjects, those in long-term care facilities, and young healthy controls.

Results: Recipient mice retained microbiota similar to donor on a synthetic diet modelled on donor type. Swapping diets altered the microbiota in the direction of the new diet. Modest changes in inflammatory parameters were noted corresponding to diet-microbiota shifts. The human dietary intervention had no global effect on microbiome diversity or composition. Bifidobacteriaceae were significantly enriched after wdietary intervention in both Community and Young Healthy while increased abundance of Ruminococcaceae and reduced Streptococcaceae were noted in Long Stay subjects.

Although the microbiome of older people is shaped by habitual diet, it is difficult to radically re-model it based on prebiotic supplementation, perhaps because responsive microbes are no longer present. Modest changes in the abundance of some species and levels of biomarkers of inflammation may nevertheless be achieved.

O228 Understanding the plant microbiome and how to make use of it in crop production

Birgit Mitter[1], Franziska Buchholz[1], Nikolaus Pfaffenbichler[1], Raheleh Sheibani-Tezerji[1], Livio Antonielli[1], Robert Junker[2], Thomas Rattei[3], Angela Sessitsch[1]

[1]AIT Austrian Institute of Technology, Center for Health & Bioresources, Bioresources Unit, Tulln, Austria
[2]University of Salzburg, Department of Ecology and Evolution, Salzburg, Austria
[3]University of Vienna, Department of Microbiology and Ecosystem Science, Division of Computational Systems Biology, Vienna, Austria

Background: Microbiomes of plants are known for their importance for healthy growth and development of the host. Similar to modern therapeutic concepts in human and veterinary medicine modifications of plant microbiomes will be an essential component of tomorrow's crop production and protection.

Objectives: In order to target the plant microbiome for improved crop production our research aim is to develop profound knowledge on plant microbiome functioning and dynamics.

Methods: We apply metagenomics to studying the microbial community composition, build-up and dynamics in crops and model plants and modern transcriptomics to elucidate the genetic basis of beneficial plant-microbe interaction and communication. Furthermore, we test the correlation between microbiome composition and crop performance and quality on the field as well as post-harvest. To translate our research to application we develop strategies for microbiome selection and modification such as an approach for changing the microbiome of crop seed embryos and concomitantly design of traits to be mediated by seed microbiomes.

Results: The plant microbiome consists of members vertically transmitted as well as recruited from the environment and it comprises a stable and opportunistic component. Plant microbiota respond to changes in plant physiology and our data show correlation between microbiome composition and plant traits during growth and post-harvest. Moreover, we demonstrated the feasibility of modifying the seed microbiome and consequently plant traits. Our data illustrate both the introduction of *P. phytofirmans* PsJN into the seeds of multiple plant species and the consequential modifications to seed microbiome composition and growth traits in wheat.

O229 Deep Impact: Sponge-associated microbes and bioactivity change with depth

Detmer Sipkema[1], Anak Agung Gede Indraningrat[1], Karin Steffen[2], Leontine Becking[3], Hauke Smidt[3], Ellen Kenchington[4], Paco Cardenas[2]

[1]*Wageningen University, Laboratory of Microbiology, Wageningen, Netherlands*
[2]*Uppsala University, Department of Medicinal Chemistry, Uppsala, Sweden*
[3]*Wageningen University, Marine Animal Ecology, Wageningen, Netherlands*
[4]*Bedford Institute of Oceanography, Department of Fisheries and Oceans, Dartmouth, Canada*

Background: Marine sponges and their microbial symbionts are leading organisms for discovery of novel bioactive compounds from nature. Yet, factors involved in shaping the microbial community and chemical potential of sponges are poorly understood. The large majority of studies on microbial composition and bioactivity of marine sponges are from shallow water, while the microbiota and bioactivity of deeper sponges are largely *mare incognita*.

Objectives: To study the impact of depth on the prokaryotic communties in marine sponges and concurrent changes in the metabolome and antimicrobial activity of sponge tissues.

Methods: By 16S rRNA gene amplicon sequencing, untargeted metabolomics and agar disc diffusion assays we assessed prokaryotic and chemical diversity and antimicrobial activity of 5 sponge species spanning a large depth range.

Results: For all sponge species studied, we find that microbiome and metabolite composition vary with depth and up to 29 % of the variation in a microbiome and up to 17% of the variation in a metabolome were attributed to depth. In addition, by investigating known bioactive compounds as well as newly identified compounds underlying the observed depth pattern, we were able to generate hypotheses about deep-sea ecology and putative bacterial producers of these compounds. For the sponge species tested the correlation between antimicrobial activity and depth was less obvious, but antibacterial activities were generally higher in shallow extracts and, conversely, higher activity against the oomycete *Saprolegnia parasitica* was observed for deeper specimens.

O230 Interplay between antibiotic persistence, tolerance and resistance in the evolution of bacteria under antibiotic tretaments

Jiafeng Liu[1], Irit Levin-Reisman[2], Irine Ronin[1], Orit Gefen[2], Nathalie Questembert-Balaban[2], Maskit Bar-Meir[3]

[1]*Hebrew University, Physics, Jerusalem, Israel*
[2]*The Hebrew University of Jerusalem, Physics, Jerusalem, Israel*
[3]*Shaare Zedek Medical Center, Jerusalem, Israel*

Background: The evolution of antibiotic resistance is a fascinating example of the versatility of bacterial evolution, as well as a burning health issue. Resistance mechanisms include efflux pumps that directly lower the intracellular drug concentration, mutations that reduce binding affinity of the drug to its target, enzymes that degrade the drug, etc. These mechanisms result in a decrease of the effective **concentration** of the antibiotic. However, bacteria were shown to be able to cope with antibiotic treatments that are supposed to kill them also using a different strategy termed "tolerance". Tolerant bacteria are not able to reduce the concentration of the antibiotic, but to make the **duration** of the treatment less effective. For example, bacteria that remain transiently dormant during the antibiotic treatment can survive because many different types of antibiotics require active growth to be able to kill.

Objectives: Our goal was to undersatdn the role played by tolerance and persistence in bacterial infections

Methods: We have developed a mathematical and experimental framework to characterize and measure the evolution of tolerance *in vitro* and *in vivo*.

Results: By following the evolution of tolerance and resistance closely, we show that tolerance evolves fast and promotes the subsequent evolution of resistance. Mathematical analysis of the way tolerance promotes the evolution of resistance reveals unexpected routes by which tolerance acts as a stepping stone for the subsequent evolution of resistance.

O231 The Type VI Secretion System of Pseudomonas aeruginosa: A Gun Loaded With Antimicrobials

Sophie A. Howard[1], Tiago Costa[1], Alain Filloux[1]

[1]*Imperial College London, Life Sciences, London, United Kingdom*

Background: *Pseudomonas aeruginosa* is an opportunistic pathogen that causes respiratory infections in cystic fibrosis patients. *P. aeruginosa* harbours the Type VI Secretion System (T6SS), a nanomachine that injects effectors into neighbouring prokaryotes or eukaryotes. The T6SS aids *P. aeruginosa*, establishing species dominance through interbacterial warfare. *P. aeruginosa* has three T6SSs, H1-3, each driving secretion of specific sets of effectors. A Hcp tube, topped with a spike and wrapped in a contractile sheath, delivers the effectors in a spear-gun-like manner. Most characterised effectors for the H1-3 are delivered via the spike. Only three characterised effectors are associated with Hcp for delivery. Tse1-3 co-purify with Hcp1, the Hcp of the H1-T6SS, and may fit within the Hcp ring with specificity. Although 25% of gram-negative bacteria possess a T6SS, Hcp-dependent delivery of effectors remains poorly understood.

Objectives: We aimed to identify a comprehensive set of Hcp-delivered effectors and characterise molecular interactions.

Methods: Investigation of Tse1-3 interaction with Hcp1 is underway to determine the interacting domains and to analyse the complex by electron microscopy. Using Hcp1-3, systematic screening pull-downs were performed to identify novel interaction partners. Engineering chimeric Tse1-3 also explores potential size limitations for inner tube delivery.

Results: We identified several novel effectors in the Hcp1-3 screen which are being investigated. These could promote *P. aeruginosa* virulence by targeting eukaryotic cells or be antibacterial. Additionally, we co-purified Hcp1 with Tse4, characterising the interaction using electron microscopy approaches along with Tse1-3. Understanding effector delivery could allow manipulation of the T6SS to deliver specific antimicrobials.

O232 Live and let die: The interaction of Listeria monocytogenes and Acanthamoeba spp. is advantageous for both parties

Mingzhen Fan[1], Lars Fieseler[2], David Drissner[3], Martin Loessner[1], Markus Schuppler[1]

[1]ETH Zurich: Institute of Food, Nutrition and Health IFNH, Department of Health Sciences and Technology, Zürich, Switzerland
[2]ZHAW: Life Sciences and Facility Management, Wädenswil, Switzerland
[3]Hochschule Albstadt-Sigmaringen, Faculty of Life Sciences, Sigmaringen, Germany

Background: *Listeria monocytogenes* is a dangerous intracellular pathogen causing listeriosis of humans. Beyond its intracellular lifestyle the bacteria are ubiquitously found in nature, where they feature a saprophytic life and share many environmental habitats with predatory protozoa.

Objectives: The aim of this study is the investigation of co-cultures of *L. monocytogenes* and acanthamoebae in order to better understand their interaction.

Methods: Co-cultures of acanthamoebae and GFP-expressing *L. monocytogenes* were investigated by confocal laser scanning microscopy in combination with a micromanipulator and electron microscopy techniques in combination with immune gold labeling.

Results: The investigation of the interaction of *L. monocytogenes* and *Acanthamoeba* spp. did not provide evidence for an intracellular persistence or multiplication in acanthamoebae. Instead, the bacteria accumulate on the surface of the trophozoites by forming large aggregates, which seem to be kept together by thin filaments. These aggregates are later phagocytosed by the trophozoites. Despite *Listeria* representing the prey, the presence of acanthamoebae supports growth of the bacteria, resulting in higher viable counts. The observation that the increasing population of *Listeria* in co-cultures represents non-motile *L. monocytogenes* indicates that cessation of motility may represent a survival strategy of *Listeria* under these conditions. In conclusion, the obtained results demonstrate that acanthamoebae do not act as environmental reservoirs for intracellular *L. monocytogenes*. However, both *Listeria monocytogenes* and acanthamoebae seem to benefit from this peculiar type of interaction. In particular cessation of motility and the ability to utilize amoebal metabolites may aid *Listeria* to avoid eradication by amoebal predation in low-nutrient environments.

O233 Design and development of synthetic macrophages to eradicate pathogenic bacteria

Zvi Hayouka[1]

[1]*The Hebrew University of Jerusalem, Institute of Biochemistry, Food Science and Nutrition, Rehovot, Israel*

Background: Resistance of bacterial pathogens to antibiotics has become a major threat to public health with drug-resistant bacteria reported to be responsible for 25,000 deaths annually in Europe alone. Hence, there is a significant and urgent need for the development of new classes of approaches to overcome the antibiotic resistance crisis. Macrophages are part of the immune system that engulf and digests microbes in a process called phagocytosis. But many pathogenic bacterial cells invade macrophages using several smart and sophisticated strategies.

Objectives: Design and devlopemnt of synthetic macrophages that will absrob and eradicate bacterial cells via contact mediated antimicrobial activity.

Methods: We have devloped a new approach to synthesize random antimicrobial peptides that showed strong and broad antimicrobial and antibiofilm activities. Random peptide mixtures contain peptides with random sequences composed of hydrophobic and cationic amino acids, totally controlled in chain length and stereochemistry. In my talk I will present our effeorts to use these novel antimicrobial agents to develop synthetic macrophages to eradicate pahogenic bacterial cells.

Results: The synthetic macrophages exhibited high bactericidal effect against gram negative and gram positive pathogenic bacteria, without any hemolytic activity of human red blood cells. Using confocal microscopy, we demonstrated the ability of the synthetic macrophages to adsorb bacteria and eradicate them. To further investigate the antimicrobial mechanism, we exposed them to *E. coli* mutant strains bearing various structures of lipopolysaccharide (LPS) layers in the outer membrane. Our results may open a new approach to tackle the antibiotic resistance crisis.

O234 Kill one to turn off many: cracking gene regulatory networks to avoid Burkholderia cenocepacia antibiotics resistance by means of multi-omics technologies

Elena Perrin[1], Marco Fondi[1], Alessio Mengoni[1], Renato Fani[1]

[1]*University of Florence, Department of Biology, Sesto Fiorentino, Italy*

Background: Antibiotics resistance (AR) and the dearth of new antimicrobials represent two of the major public health problems of this century. One possible strategy to overtake this problem could be avoid the development of AR and restore the effectiveness of existing antibiotics. However, this strategy is not of simple application, due to the fact that the AR development is a complex phenomenon. In this context, the availability of quantitative, predictive models might strongly facilitate the formulation of strategies to limit the efficacy and evolvability of drug resistance.

Objectives: The aim of this work is to reconstruct and model the regulatory network of *Burkholderia* RND systems (one of the main causes of AR in Gram-negative bacteria), using *Burkholderia cenocepacia* J2315 as a model system.

Methods: We are combining different wet- lab experiments, qPCR, transcriptomics and genomics analysis, to obtain all the relevant -omics information for the reconstruction of a model of the regulatory network(s) of RND operons in *Burkholderia* cells.

Results: After reconstructing the regulative circuit model, expression data obtained through qPCR and RNA- seq, will be used to simulate the global changes in genes expression that take place in the presence of a given stimulus, in order to identify the most important genes involved in stress response and antibiotics resistance. These genes are the target to be "killed" to avoid the activation of the mechanisms of resistance.

O235 Combining micro and nanoscopic imaging methods: a new look at antibiotic activity

Rym Boudjemaa[1], Clément Cabriel[2], Nicolas Bourg[1], Romain Briandet[3], Sandrine Lévêque-Fort[2], Karine Steenkeste[2], Christian Marliere[2], Marie-Pierre Fontaine-Aupart[2]

[1]*Abbelight, Paris, France*
[2]*INSTITUT DES SCIENCES MOLECULAIRES D'ORSAY (ISMO), Orsay, France*
[3]*INRA, Jouy-en-Josas, France*

Background: Among current clinical antibiotics used to treat bacterial infections, very few enable long-term successful treatment, in part due to the well-described genetic-based resistance. In addition, the physiological modifications occurring when bacteria stick to biotic or abiotic surfaces and further form biofilms are strongly involved in antibiotics failure.

Objectives: The goal of this work was to visualize the activity/inactivity of antibiotics on bacteria, from a micro to a nanoscopic imaging perspective.

Methods: We focused the study on *Staphylococcus aureus* under daptomycin exposure, a last-resort antibiotic. The advanced imaging methods, optimized for live bacteria studies, included (i) three-dimensional single molecule super-resolution imaging used to ascertain that the antibiotic effectively reached its biological target, (ii) confocal laser scanning microscopy to probe antibiotic (in)activity, and (iii) atomic force microscopy to highlight the impact of antibiotic action on the topographical and mechanical properties of single bacterial cells surface.

Conclusions: Importantly, the combination of these methods was necessary to reveal that daptomycin could effectively reach its biological target in live *S. aureus* whatever the bacterial physiological state. By contrast, the antibiotic oligomerization within the bacterial membrane and subsequent bacterial surface damage were dependent on bacterial cells physiology.

O236 Transfer dynamics of the antibiotic resistance plasmid pOXA-48 in a hospital

Ricardo Leon-Sampedro[1], Javier de la Fuente[1], Cristina Díaz-Agero[1], Thomas Crellen[2], Patrick Musicha[2], Jerónimo Rodríguez-Beltrán[1], Marta Hernández García[1], Nieves López Fresneña[1], Patricia Ruiz-Garbajosa[1], Rafael Canton Moreno[1], Ben Cooper[2], Alvaro San Millan[1]

[1]Ramon y Cajal Institute for Health Research (IRYCIS), Microbiology, Madrid, Spain
[2]Mahidol Oxford Tropical Medicine Research Unit, Mathematical and Economic Modelling (MAEMOD), Bangkok, Thailand

Background: Plasmids drive the horizontal transfer (HT) of resistance genes between pathogenic bacteria by conjugation, playing a pivotal role in the evolution of antibiotic resistance. During the last decade carbapenem-degrading enzymes (carbapenemases) have emerged as a main concern for the treatment of clinically relevant bacteria, and pOXA48 is one of the most prevalent carbapenemase-carrying plasmids in Enterobacteriaceae.

Objectives: Our objective was to characterize the inter- and intra-patient transmission routes of pOXA-48 in a main University Hospital.

Methods: We analysed an enterobacteria strain collection isolated from 15,556 faecal samples from 11,285 hospitalized patients in four wards of our hospital from 2014 to 2016. We used Ilumina-HiSeq4000 to perform whole genome sequencing of the 171 pOXA-48-carrying enterobacteria isolated during this period from 135 patients. We combined epidemiological data, genome sequences and Bayesian hierarchical models to estimate the daily probability of acquiring the pOXA-48 plasmid and to identify the plasmid transmission routes (using Bitrugs, SCOTTI and outbreaker2).

Results: We demonstrated that inter-patient transmission of pOXA-48 is mediated mainly by *K. pneumoniae* (*Kpn*). In addition, our results suggest that after colonization, pOXA-48-carrying *Kpn* transfer pOXA-48 towards other enterobacteria present in the gut of the patients. We identified specific *Kpn* clones (ST11) as responsible for most of the inter-patient transmission events, producing discrete outbreaks inside the hospital. Furthermore, we found superspreader patients acting as dissemination nodes for *Kpn*/pOXA-48. In conclusion, we used a novel approach to characterize the inter- and intra-patient transmission routes of an epidemiologically relevant antibiotic resistance plasmid in a real clinical scenario.

O237 Novel food grade microbial cell factories for aroma production

Eddy J. Smid[1], Judith C. M. Wolkers-Rooijackers[1], Tjakko Abee[1], Oscar van Mastrigt[1]

[1]*Wageningen University, Laboratory of Food Microbiology, Netherlands*

Background: The knowledge of microbial physiology and metabolism is mostly derived from studies of fast growing cultures of microbes. In nature, most microbes are continuously exposed to energy limitation. Consequently, they have evolved strategies to combat unfavourable conditions. Due to the use of nutrient rich media and focus on exponential growth in microbiological research, we still have a limited understanding of the metabolism of microbes coping with severe nutrient limitation. This unknown territory of microbial behaviour potentially offers possibilities for developing new biotechnological applications of common microbial workhorses.

Objectives: The aim of this study was to explore the properties of cell cultures under severe nutrient limitation and exploit these properties for developing new biotechnological processes. Here, we focus on the production of dairy related aroma compounds by *Lactococcus lactis* growing at near zero-growth rates.

Methods: The metabolic behaviour of *L. lactis* was studied at extremely low growth rates imposed by prolonged retentostat cultivation. Maintenance energy requirement, metabolite production, stress resistance, viability, plasmid content and morphology of the retentostat cultivated cells were investigated and compared to relevant control cultures.

Results: We demonstrated the formation of *viable but not culturable L. lactis* cells in response to near-zero growth rates. Moreover, the maintenance requirement was found to decrease 7-fold at growth rates below 0.0025 h^{-1}. Finally, we discovered that the production of volatile aroma compounds of retentostat cultures of *L. lactis* resembled the aroma profiles of ripened cheese, demonstrating new options for biotechnological production of complex aroma blocks.

O238 Phage-host interactions in dairy fermentations: science-based selection of starter cultures

Douwe van Sinderen[1]

[1]*University College Cork, School of Microbiology, Cork, Ireland*

Background: Dairy fermentations require specific lactic acid bacterial starter cultures to acidify milk and endow its final products with particular organoleptic properties. Such starter cultures are therefore crucial to ensure rapid milk acidification, as well as associated product quality and consistency. Phage predation is a common and persistent problem in such dairy fermentations.

Objectives: A thorough understanding of phages that infect the main lactic acid bacterial species used, *Lactococcus lactis* and *Streptococcus thermophilus*, is essential in order to appreciate the manner by which they recognize and infect their hosts, and to come up with rational approaches to mitigate dairy phage infections.

Methods and Results: This talk will provide an up-to-date view of dairy phages, their host recognition mechanism, and the saccharidic molecules they target on the lactic acid bacterial surface. The generated knowledge has allowed the classification of hosts into distinct groups based on their surface glycans, which in turn predict their sensitivity to various phages.

O239 Bridging food and gut health: prevalence and functional traits of lactic acid bacteria and probiotics in the gut microbiome

Edoardo Pasolli[1], Francesca De Filippis[1], Paolo Manghi[2], Nicola Segata[2], Danilo Ercolini[1]

[1]*University of Naples Federico II, Agricultural Sciences, Portici, Italy*
[2]*University of Trento, Italy*

Background: Functional foods (FF) are enhanced to furnish health benefits when consumed regularly. Several FF are enriched in probiotic microorganisms. Fermented foods are recognized as potentially functional because they can deliver live lactic acid bacteria (LAB) that may have probiotic properties and positively affect human gut health. LAB are widespread in fermented foods, they arise from raw materials, environment of production and from addition of starter or probiotic cultures.

It is still unknown how much of the LAB and probiotics we ingest with food becomes actually part of the gut microbiome and contribute to gut homeostasis.

Objectives: To provide a large-scale investigation on the prevalence and functional properties of LAB and probiotics in the gut microbiome of populations spanning ages, countries, and lifestyles.

Methods: We leveraged ~8500 metagenomes from public available repositories to search for probiotic and food-associated LAB species in the human gut microbiome. This was done using a compendium of tools based on taxonomic profiling, strain-level analysis, and single-sample metagenomic assembly.

Results: We identified >20 probiotic and food-associated LAB species with a prevalence > 0.5% in the human gut of the general population. *Streptococcus thermophilus* (32% of prevalence) and species of the genus *Lactobacillus*, which are widespread in fermented foods, have higher prevalence and relative abundance in westernized compared to non-westernized populations. By contrast, heterofermentative *Leuconostoc* and *Weissella* species were more typical - although at low abundances - of non-western populations. Functional traits were investigated by comparative genome-wide analysis of >500 genomes newly reconstructed from the gut microbiome.

O240 Beerome: predicting sensory responses to beer with machine learning methods

Supinya Piampongsant[1;2;3], Miguel Roncoroni[1;2;3], Christophe Vanderaa[4], Tom Wenseleers[4], Beatriz Herrera[1;2;3], Lukasz Kreft[5], Ruben Wauters[1;2;3], Alexander Botzki[5], Kevin Verstrepen[1;2;3]

[1]VIB, KU Leuven Center for Microbiology, Heverlee-Leuven, Belgium
[2]University of Leuven, CMPG Laboratory of Genetics and Genomics, Heverlee-Leuven, Belgium
[3]Leuven Institute for Beer Research, Heverlee-Leuven, Belgium
[4]University of Leuven, Laboratory of Socioecology and Social Evolution
[5]VIB, Bioinformatics Core, Belgium

Background: After thousands of years of beer brewing, we now have a significant grasp on the science of brewing and the chemical composition of beers. Despite all this information, we are still unable to accurately connect a beer's chemical profile to its sensory perception and overall appreciation by consumers. Indeed, while sensorial properties of single beer constituents are often known, complex interactions between them (masking, temporal, synergistic effects, and physical interactions) make accurate predictions of overall beer perception nearly impossible.

Objectives: In this project, we used machine learning to develop a collection of models that accurately predict sensory responses to beer chemical compositions.

Methods: We have characterized the chemical profiles of 250 commercial Belgian beers, including the alcohol content, color, pH, caloric value, and concentrations of compounds from yeast, hops, malt, spices, bacteria. The beers' sensory qualities were assessed by fifteen trained panelists in blind tasting sessions. Ratings and review texts from more than 6,000 users were further collected from Ratebeer, a publicly accessible beer rating website. State-of-the-art algorithms were then employed to predict beer flavors, aromas, and overall appreciation from chemical parameters for both the expert panel and public populations.

Results: We are able to predict the sensorial properties of beer at a higher accuracy than previously achieved. Our models yield insights into the chemical parameters that dominate our sensory experiences, highlighting the important roles that microbes play in producing them.

O241 Genome sequencing and food safety

Leen Baert[1]

[1]*Nestlé Research*

Background: Whole genome sequencing (WGS) is becoming the gold standard subtyping technique in foodborne outbreak investigations and surveillance. WGS allows the detection of smaller foodborne outbreaks by its ability to link clinical cases with high resolution that would not have been possible with previous subtyping methods such as Pulsed Field Gel Electrophoresis (PFGE). Besides the benefits, the introduction of WGS in food safety and public health brings along numerous new challenges. One of the challenges is the sharing of sequence data that is hampered by several aspects such as political barriers, legislation and data protection. Additionally, a common language on the utility of WGS between all stakeholders within the farm to fork chain is crucial to understand better transmission pathways with an aim to take appropriate actions. In many instances, the cost of WGS is underestimated by promising the multi-purpose analysis (e.g. virulence, antimicrobial resistance characterization at the same time). However, for many stakeholders this additional characterization is not necessarily required in every case. Since WGS analysis requires not only microbiology knowledge but also genomics and bioinformatics expertise, data interpretation is not straightforward and should be carefully done in combination with strong metadata/epidemiological information. Instead of sequencing an isolate, sequencing samples directly without culturing, i.e. metagenomics is gaining interest especially for clinical diagnostics. The absence of live cultures will bring new challenges on data interpretation.

Objectives: The aim of the workshop is to address the benefits and challenges of WGS and metagenomics analysis for food safety.

O242 Tracing microbial contamination sources in the meat production chain

Benjamin Zwirzitz[1;2], Stefanie U. Wetzels[1;2], Emmanuel D Dixon[1], Isabel Rabanser[1], Sarah Thalguter[1;2], Monika Dzieciol[1], Martin Wagner[1], Evelyne Mann[1]

[1]*Institute of Milk Hygiene, Milk Technology and Food Science, Department for Farm Animal and Public Health in Veterinary Medicine, Vienna, Austria*
[2]*FFoQSI GmbH, Austria*

Background: The food industry faces major and continuing challenges in trying to lower the extent of contamination by pathogenic or spoilage bacteria during primary processing. While pork accounts for half of the annual meat production in the European Union and is one of the main causative agents of food-borne disease outbreaks, little is known about the actual microbial diversity within pork processing plants.

Objectives: Our approach was to explore and investigate the possible sites of microbial transmission in a slaughterhouse and meat cutting plant to effectively reduce the risk of cross-contamination. Specifically, we investigated what proportion of bacteria on meat are not animal-associated and are therefore transmitted during cutting via personnel, equipment, machines, or the slaughter environment.

Methods: Samples were taken from 12 pigs at different stages during processing as well as from multiple sites throughout the facility environment (258 samples total). Using a hybrid 16S rRNA gene sequencing approach (combining Illumina Miseq and Pacbio Sequel), we aimed to classify the detected taxa up to the species level, while gaining information about the full diversity of microorganisms.

Results: We were able to identify key sources of bacterial contamination and create a facility-specific transmission map of bacterial flow. For example, the *Moraxella* spp. were transmitted primarily from the polishing tunnel water, whereas *Pseudomonas* spp. and *Brochothrix* spp. were transmitted from the conveyor belt. Overall, we expand our knowledge about the transmission routes for microbial contamination in meat processing, while highlighting possibilities to improve hygiene standards in the food industry.

O243 Isolation and genetic/phenotypic characterisation of Listeria monocytogenes biofilm specialists

Natalia Crespo[1], Eva Dax[1], Heidy den Besten [1], Tjakko Abee[1]

[1]*Wageningen University & Research, Food Microbiology, Wageningen, Netherlands*

Background: *Listeria monocytogenes* is a foodborne pathogen capable of forming biofilms on the surface of food-processing equipment.

Objectives: the aim of this study was to identify genetic and/or phenotypic traits that may play a role in biofilm formation.

Methods: a bead-based experimental evolution system was developed. Cells were incubated with a sterile bead for two days, washed and transferred to fresh media containing an oppositely-coloured bead. Both beads were incubated together, ensuring a cycle of dispersal, colonisation and biofilm formation that allowed for the selection of good biofilm formers. After six weeks the beads were sampled and the biofilm formation ability of the obtained isolates was tested via crystal violet staining. Selected isolates were sent for genome sequencing, and a SNP analysis was performed between the ancestral (AN) and the evolved isolates (EV). Further characterisation of the isolates is ongoing.

Results: the EV isolates produced five times more biofilm than the AN, reaching its peak after 6 hours of incubation, which suggests an increased attachment ability. The SNP analysis of one of the EV isolates showed a deletion in *virB* that leads to an earlier stop codon. *VirB* is part of a two-component and ABC transporter system that has been previously linked to biofilm formation in *L. monocytogenes*, although the mechanism by which this happens is still unknown. We hypothesise that it might be linked to differences in the expression of certain genes controlled by the *virR* regulon, such as those involved in cell surface modifications and teichoic acid production.

O244 Whole-genome-based phylogeny of Bacillus cytotoxicus reveals different clades within the species and provides clues on ecology and evolution

Marc Stevens[1], Taurai Tasara[1], Jochen Klumpp[2], Monika Ehling-Schulz[3], Sophia Johler[1]

[1]*University of Zurich, Institute for Food Safety and Hygiene*
[2]*Institute for Food, Nutrition and Health, ETH Zurich*
[3]*University of Veterinary Medicine Vienna*

Background: *Bacillus cytotoxicus* is a member of the *Bacillus cereus* group linked to fatal cases of diarrheal disease. Information on *B. cytotoxicus* is very limited; in particular comprehensive genomic data is lacking.

Objectives: We aimed to apply a genomic approach to characterize *B. cytotoxicus* and decipher its population structure.

Methods: Complete genomes of ten *B. cytotoxicus* were sequenced and compared to the four publicly available full *B. cytotoxicus* genomes and genomes of other *B. cereus* group members.

Results: Average nucleotide identity, core genome, and pan genome clustering resulted in clear distinction of *B. cytotoxicus* strains from other strains of the *B. cereus* group. Genomic content analyses showed that a hydroxyphenylalanine operon is present in *B. cytotoxicus,* but absent in all other members of the *B. cereus* group. It enables degradation of aromatic compounds to succinate and pyruvate and was likely acquired from another *Bacillus* species. It allows for utilization of tyrosine and might have given a *B. cytotoxicus* ancestor an evolutionary advantage resulting in species differentiation. Plasmid content showed that *B. cytotoxicus* is flexible in exchanging genes, allowing for quick adaptation to the environment. Genome-based phylogenetic analyses divided the *B. cytotoxicus* strains into four clades that also differed in virulence gene content.

O245 Characterisation of production strains of food enzymes and its relevance in safety assessment

Jaime Aguilera[1], Magdalena Andryszkiewicz[1], Giovanni Bernasconi[1], Christine Horn[1], Natalia Kovalkovicova[1], Yi Liu[1], Joaquim Maia[1], Sandra Rainieri [1], Claudia Roncancio-Peña[1], Ana Gomes[1]

[1]*European Food Safety Authority, FIP Unit*

Background: More than 300 food enzymes are marketed in the EU, the vast majority obtained from microorganisms. For regulatory reasons, all must undergo a risk assessment by the European Food Safety Authority (EFSA).

Objectives: The characterisation of the production strain is paramount for this assessment. It involves its taxonomical identification, evaluation of its pathogenic and/or toxigenic potential, its antimicrobial resistance (AMR), and any genetic modification.

Methods: EFSA proposes Whole Genome Sequence (WGS) analysis as the preferred methodology for the characterisation. There are standards for its use in bacterial identification and platforms also exist for fungi. Relevant databases are available to search for sequences involved in virulence and AMR. Genetic modifications can be reliably characterised by comparison between the genomes of the modified and parent strains. The value of this approach has been recently discussed.

Results: The result of this characterisation greatly influences the assessment of the enzyme. Depending on the pathogenic and/or AMR profile of the strain, it may be necessary to test whether viable cells remain in the product. If the strain carries acquired AMR genes, the presence of gene-size fragments of DNA in the food enzyme should be studied (mandatory for genetically modified strains). The characterisation of the strain also determines whether toxicological studies of other enzyme obtained from a relative strain can be used to support safety, and even whether toxicological studies are at all needed. For example, strains qualifying for the so-called Qualified Presumption of Safety (QPS) are considered safe without the need of further testing.

O246 A polyphasic methodological approach reveals low clostridial abundance despite apparent cheese spoilage

Johanna Brändle[1;2], Vera Fraberger[1], Julia Berta[1], Edoardo Puglisi[3], Mansooreh Jami[1], Wolfgang Kneifel[1], Konrad J. Domig[1]

[1]BOKU - Institute of Food Science, Department of Food Science and Technology, Vienna, Austria
[2]FFoQSI GmbH - Austrian Competence Centre for Feed and Food Quality, Safety and Innovation
[3]DiSTAS - Facoltà di Scienze agrarie, alimentari e ambientali, Sede di Piacenza, Dipartimento di Scienze e tecnologie alimentari per una filiera agro-alimentare sostenibile

Background: Clostridia cause severe sensory and textural defects in hard and semi-hard cheese. Due to excessive gas production during ripening, cheese spoilage caused by clostridia is called ”late-blowing”. Although this spoilage phenomenon is a long-standing problem of economic importance for dairy processors, limited data on clostridia in cheese exists that would allow some tailored intervention.

Objectives: The aim of this study was to identify and quantify clostridia in late-blown cheese to improve our understanding of clostridia involved in cheese spoilage.

Methods: Fifty-one cheeses, which showed symptoms of late-blowing, were analysed using the following culture-dependent methods: MPN (Most probable number) using Bryant and Burkey broth, selective enrichment, pre-screening for clostridia using cluster-specific PCR and species identification using 16S rDNA sequencing. Additionally, each cheese was analysed using the following culture-independent methods: species-specific real-time PCR, PCR-DGGE (polymerase chain reaction – denaturing gradient gel electrophoresis) and Illumina 16S rDNA sequencing.

Results: Using the MPN method, high clostridial spore levels were detected in only 14 % of the cheeses. However, clostridial isolates were obtained from 73% of the samples. In contrast to other studies, an extremely low diversity of Clostridium species was observed as the most frequently isolated species C. tyrobutyricum represented 96% of the total clostridial isolates. Real-Time PCR and PCR-DGGE were the most sensitive methods yielding positive results for C. tyrobutyricum in the majority of cheese samples. Interestingly, Illumina 16S rDNA sequencing revealed relative abundances of clostridia below the detection limit despite apparent cheese spoilage.

O247 Analysis of dairy products and their processing environments as a reservoir of determinants of antimicrobial resistance through functional metagenomics

Elena Oniciuc[1], Calum Walsh[2], Amal Awad[3], Laura Coughlan[2], Cezara Simon[1], Paul Cotter[2;4], Avelino Álvarez Ordóñez[1;5]

[1]*Universidad De Leon, Food Hygiene and Technology, León, Spain*
[2]*Teagasc Food Research Centre, Fermoy, Ireland*
[3]*Mansoura University, Mansoura, Egypt*
[4]*APC Microbiome Ireland, Cork, Ireland*
[5]*Universidad De Leon, Institute of Food Science and Technology, León, Spain*

Background: Culture-independent functional metagenomics allows studying complex microbial ecosystems. Food-related microorganisms can act as reservoirs of antimicrobial resistance genes (ARGs), which can also be transferred horizontally to food-borne pathogens.

Objectives: To identify ARGs present in the microbiota of raw milk, dairy products and their processing environments using functional metagenomics.

Methods: A metagenomic library was constructed with ~22,000 recombinant *E. coli* clones containing microbial DNA from the above mentioned reservoirs. The library was replicated on Luria Bertani agar plates supplemented with ampicillin, gentamycin, ciprofloxacin, tetracycline, cefotaxime and colistin. Fosmid DNA from resistant clones was subjected to restriction analyses and then sequenced to identify ARGs responsible for the phenotypes observed. Besides, all inserts were sequenced through high throughput sequencing (HTS) and taxonomic and functional predictions were made.

Results: The rate of resistant clones was 0.02% for raw milk, 0.28% for raw milk cheeses and 0.04% for processing environments, including the following: ciprofloxacin (1), gentamicin (1), tetracycline (2), cefotaxime (3) and ampicillin (25). HTS analyses evidenced differences in taxonomic composition and ARGs presence between the different reservoirs. Actinobacteria and Firmicutes were the most abundant phyla, followed by Proteobacteria and Euryarchaeota. The most abundant ARGs were multiple-resistance genes, but ARGs to aminoglycosides, beta-lactams, bacitracin, sulfonamides and trimethoprim were also found. Most of the ARGs were assigned to *Ralstonia solanacearum* and other unclassified species. *Campylobacter* spp. and *Clostridium* spp. were also important reservoirs. In raw milk and raw milk cheeses, the presence of trimethoprim ARGs associated mainly with *Salmonella enterica* and *E. coli* was notable.

O248 Clinical microbiology laboratory evolution: to centralize or not to centralize, that is the question!

Maurizio Sanguinetti[1]

[1]*Fondazione Policlinico Universitario "A. Gemelli", Department of Laboratory Medicine and Infectious Diseases, Rome, Italy*

Infectious disease management essentially consists in identifying the microbial cause(s) of an infection, initiating if necessary antimicrobial therapy against microbes and controlling host reactions to infection. In clinical microbiology, the turnaround time of the diagnostic cycle (>24 hours) often leads to unnecessary suffering and deaths; approaches to relieve this burden include rapid diagnostic procedures and more efficient transmission or interpretation of molecular microbiology results. Although rapid nucleic acid-based diagnostic testing has demonstrated that it can affect the transmission of hospital-acquired infections, we believe that such life-saving procedures should be performed closer to the patient, in dedicated 24/7 laboratories of healthcare institutions, or ideally at point of care. While personalized medicine generally aims at interrogating the genomic information of a patient, drug metabolism polymorphisms, for example, to guide drug choice and dosage, personalized medicine concepts are applicable in infectious diseases for the (rapid) identification of a disease-causing microbe and determination of its antimicrobial resistance profile, to guide an appropriate antimicrobial treatment for the proper management of the patient. The implementation of rapid diagnostic tests for infectious diseases will require acceptance by medical authorities, new technological and communication platforms, as well as reimbursement practices such that time- and life-saving procedures become available to the largest number of patients.

O249 Automation in clinical microbiology laboratory: current status and future developments

Katja Seme[1]

[1]*University of Ljubljana, Faculty of Medicine, Ljubljana, Slovenia*

Background: Automated immunoassays have been in clinical microbiology laboratories for decades and current analyzers process samples in batches or by random access and can run broad range of serological assays for several pathogens. Similarly, in molecular diagnostics several platforms fully automate nucleic acid extraction, amplification, and detection in closed single-use devices and enable detection of single or several pathogens/targets/resistance markers in single specimen with short turn-around-time. In contrast, automation was not considered applicable in bacteriology for several reasons.

Objectives: Recently, growing shortages of trained personnel, a growing demand for improved quality, and two very important technological innovations: the introduction of liquid-based swab transport devices and the emergence of MALDI-TOF technology have triggered the development of automated solutions designed for bacteriology.

Methods: The automation solutions can be currently divided into automated specimen processors and systems that offer partial or total laboratory automation. Both partial and complete lab automation are composed of specimen processors and incubators with digital imaging that are connected by a conveyor system, but only one system provides integrated workbenches with a two-way track system for plate delivery.

Results: Automated processing instruments produce more isolated colonies, exhibit enhanced reproducibility and provide decreased hands-on plating time than manual plating. The higher yield of isolated colonies obtained by automated systems compared to manual inoculation can greatly decrease the requirement for subculturing and result in a significant decrease in time to result, laboratory workload and laboratory costs.

O250 New concepts of molecular and phenotypic detection of antimicrobial ressitance

Frederic Laurent[1]

[1]*Institute for infectious Agents, Bacteriology, Lyon, France*

Background: The accurate detection of antimicrobial resistances is pivotal in clinical microbiology, but in the context of "one health" health approach this detection is now used in veterinary and environmental bacteriology. The multiplicity of resistance mechanisms and their combination in one single strains as well as the low expression of some mechanisms contribute to the difficulty of the rapid and precise determination of the resistance profiles and identification of the mechanism(s) involved.

Objectives: To describe the new concepts of phenotypic and molecular detection of antimicrobial resistance.

Methods: The presentation will present an overview of the most recent approaches developed and used in bacteriology laboratory to determine the mechanisms of resistance in Gram positive and Gram negative bacteria.

Results: The techniques using new molecular approaches including multitarget panel technology will be explained based on selective examples.

The new phenotypic approaches based on hydrolytic activity of bacteria on antibiotics, the metabolic activity of bacteria in presence/absence of antibiotics or direct detection of proteins involved in resistance mechanisms using maldi-tof, pH changes or immunochromatography will be explored.

O251 Next generation sequencing: first diagnostic one-stop show in clinical microbiology

John W. A. Rossen[1;2], Monika Chlebowicz[1], Silvia García-Cobos[1], Alida Veloo[1], Mirjam Kooistra-Smid[1;3], Alexander W. Friedrich[1], Natacha Couto[1]

[1]University of Groningen, University Medical Center Groningen, Department of Medical Microbiology and Infection Prevention, Groningen, Netherlands
[2]ESCMID Study Group for Genomic and Molecular Diagnostics
[3]Certe, Department of Medical Microbiology, Groningen, Netherlands

Background: Current molecular diagnostics of human pathogens provide limited information that is often not sufficient for outbreak and transmission investigation.

Objectives: In this presentation an overview of the use of next generation sequencing in clinical microbiology and infection prevention will be given.

Methods: Next generation sequencing (NGS) determines the DNA sequence of a complete bacterial genome in a single sequence run, and from these data, information on resistance and virulence, as well as information for typing is obtained, useful for outbreak investigation.

Results: The obtained genome data can be further used for the development of an outbreak-specific screening test. Like every new technology adopted in microbiology, the integration of NGS into clinical and routine workflows must be carefully managed. As the microbiology laboratories have to adhere to various national and international regulations and criteria for their accreditation, quality control issues for using WGS in microbiology, including the importance of proficiency testing, are presented. In addition, applications of NGS in the clinical setting are discussed, such as outbreak management, molecular case finding, characterization and surveillance of pathogens, rapid identification of bacteria using the 16S-23S rRNA region, taxonomy, and metagenomics approaches on clinical samples. Finally, we share our vision on the use of NGS in personalised microbiology in the near future, pointing out specific requirements.

O252 Characterisation of Aurodox, A Type III Secretion System Inhibitor from *Streptomyces*

Rebecca McHugh[1;2], Paul Hoskisson[1], Andrew Roe[3]

[1]*Strathclyde Institute of Pharmacy and Biomedical Sciences, Microbiology, Glasgow, United Kingdom*
[2]*Institute of Infection, Immunity and Inflammation, Bacteriology, Glasgow, United Kingdom*
[2]*Institute of Infection, Immunity and Inflammation, Bacteriology, Glasgow, United Kingdom*

Background: Enteropathogenic *Escherichia coli* (EPEC) and Enterohemorrhagic *Escherichia coli* (EHEC) are enteric pathogens which utilize a Type III Secretion System (T3SS) for host colonization. Aurodox, a specialised metabolite from the soil bacterium *Streptomyces goldiniensis,* has been shown to inhibit the EPEC Type III Secretion System (T3SS), and hence the compound has been nominated as a potential anti-virulence drug for these infections, for which treatment with traditional antibiotics is not recommended.

Objectives: We aimed to characterize the effects of Aurodox on carriers of a homologous Type III Secretion System encoded for by the Locus of Enterocyte Effacement (LEE) pathogenicity island, including *Citrobacter rodentium* and Enterohemorrhagic *Escherichia coli* (EHEC), which has high prevalence in Scotland. To further assess the utility of this compound as an anti-virulence drug, we aimed to gain further understanding of the effect of Aurodox on the ability of EHEC to colonize epithelial cells. In addition, we aimed to characterize the molecular mechanism of T3SS inhibition by Aurodox.

Methods: Cell infection assays were used to determine the effect of Aurodox on colonisation efficiency. Whole transcriptome analysis and GFP-reporter assays were used to determine the effect of Aurodox on T3S and bacterial SOS response expression in EHEC.

Results: Our results support a model where Aurodox transcriptionally downregulates the expression of the Locus of Enterocyte Effacement (LEE) pathogenicity island- which encodes for the T3SS, acting via its master regulator, *Ler.* Thus, reducing EHEC colonisation efficiency by >5000 fold.

O253 Small RNAs in the control of bacteriophage development

Grzegorz Wegrzyn[1]

[1]*University of Gdansk, Department of Molecular Biology, Gdansk, Poland*

Small non-coding RNAs are known to play important regulatory roles in both eukaryotic and prokaryotic cells. Bacteriophages are not an exception, and various small RNAs have been discovered to control phage development. The best examples are bacteriophage lambda and related phages, called lambdoid phages, and RNA molecules regulating various processes. Until recently, only two small regulatory RNAs encoded by lambdoid bacteriophages were known. These transcripts are derived from *p*aQ and *p*O promoters. The former one is supposed to act as an antisense RNA for expression of the *Q* gene, encoding a transcription antitermination protein. The latter transcript, called oop RNA, derives from the *p*O promoter which has been demonstrated to be important for both regulation of phage development and control of DNA replication. Namely, the *p*O-derived transcript is an antisense RNA for expression of the *c*II gene, and *p*O is a part of a dual promoter system responsible for regulation of initiation of DNA synthesis from the *ori* region. Recent studies identified that microRNA molecules can have biological functions not only in eukaryotes but also in prokaryotic cells. The first functional prokaryotic microRNA was found to be encoded by lambdoid bacteriophage, and to play a role in the regulation of phage development through modulating gene expression.

O254 Interesting properties of Yersinia bacteriophages

Mikael Skurnik[1]

[1]*University of Helsinki, Department of Bacteriology and Immunology, Helsinki, Finland*

I will present data on phages ϕEV1 (a dwarf myovirus), ϕR1-37 (a giant myovirus) and YerA41 (a myovirus), infecting *Yersinia pestis, Y. enterocolitica* and *Y. ruckeri,* respectively. ϕEV1 was isolated in 1999 from sewage sample in Turku, Finland, using the *Y. pestis* vaccine strain EV-76 as a host. Genome sequencing revealed novel features in its physical ends. ϕR1-37 was isolated in 1988, also from the Turku Cty sewage, and it has all thymidines in its DNA replaced by deoxyuridine (dU). It has a rather broad host range among *Yersinia* species, and uses in various hosts completely different lipopolysaccharide structures as receptors. YerA41, isolated in 1984 from city sewage in Guelph, Ontario, Canada (Stevenson, AEM, 1984), was sent to us by Hans-Wolfgang Ackermann. Several attempts to determine its DNA-sequence using traditional and next generation sequencing technologies failed indicating that the phage genome nucleotides carry a novel modification rendering the phage DNA unsuitable as a template to ordinary DNA-polymerases. Also, all the used restriction enzymes failed to digest the DNA. To get hold of the nucleotide sequence of YerA41 we isolated total RNA from phage-infected *Y. ruckeri* cells at different time points post-infection (0 – 90 min) and carried out RNA-sequencing. This resulted in 9 phage-specific contigs of a total of 145 kb. Annotation revealed 196 potential genes with only 33 having homologs in the databases. Studies to elucidate the nucleotide modifications are under way.

O255 Phage repurposing: noval targets for phage therapy

Andrzej Gorski[1;2], Ryszard Międzybrodzki[1;2], Krystyna Dąbrowska[1], Ewa Jończyk-Matysiak[1], Jan Borysowski[1], Marzena-Łusiak Szelachowska[1], Beata Weber-Dąbrowska[1]

[1]*Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Poland*
[2]*The Medical University of Warsaw, Poland*

The real value of anti-bacterial phage therapy assessed according to the standards of evidence-based medicine awaits confirmation by clinical trials. However, recent findings strongly suggest that phages are not only mere "bacteria eaters" but have much broader potential for use in medicine. Accumulating data indicate that phages may also interact with eukaryotic cells including cells of immune system causing anti-inflammatory and immunomodulating action. The fact that phages may combine their anti-bacterial action with anti-inflammatory and immunoregulatory effects suggests that their potential therapeutic applications may acquire new dimensions well beyond mere elimination of bacteria. This new concept of phage therapy could offer new perspectives for treating so far poorly controlled disorders in which there exists no targeted therapy or the currently available therapies have significant side effects. Sepsis constitutes an excellent example of the potential of phage therapy in this clinical setting where phages can eliminate offending bacteria and endotoxin and downregulate inflammation (sepsis is believed to be a complex disorder where an uncontrolled inflammatory response to bacterial infection constitutes a hallmark of that pathology). Moreover, data have been accumulating suggesting that phage therapy could also target non-bacterial infections as well as allergy, autoimmune disorders, allograft rejection and graft-versus-host disease. Thus, the potential for broader non-bacterial application of PT is evident and it is certainly worthy of further studies.

O256 Are bacteria using phages in their own profit?

Maite Muniesa [1]

[1]*University of Barcelona, Genetics, Microbiology and Statistics, Barcelona, Spain*

Background: Bacterial chromosome features numerous prophages, many of which encode virulence-related genes, providing some advantage for their bacterial host. In contrast, other prophages do not encode any known beneficial gene, are unable to generate virions (cryptic phages) or have lost part of their genome, (remnants or ancient prophages). The issue of why these remnant sequences prevail in the bacterial chromosome is intriguing. If the information these fragments contain is incomplete, and bacterial cells tend to eliminate any information not directly beneficial for their adaptation, why do they accumulate so many prophages?

Objectives: Using *Escherichia coli* as a model and focusing on antibiotic resistance genes (ARG), we attempt to demonstrate that *E. coli* generate phage particles containing ARG as a part of a mechanism that bacteria use to spread its own DNA.

Methods: Prophages were induced from *E. coli* isolates possessing ARGs with mitomycin C. Phage particles in the cultures were carefully purified to avoid bacterial contamination by filtration, chloroform, DNase treatment and CsCl density gradients. ARGs were investigated in the particles that were confirmed as phages by proteomics and microscopy. Finally, infectivity of the phage particles and ARG transduction were assayed.

Results: Phage capsids packaging ARGs were generated from *E. coli*, however these ARGs were not located in a prophage genome. These particles contained bacterial DNA instead of phage DNA and did not generate plaques of lysis. Our investigation supports the hypothesis that prophages in bacterial chromosomes are used by bacteria as a mechanism to disseminate their own genetic content.

O257 Development of a safe whole-cell Bordetella pertussis vaccine: application of lipid A engineering

Jesús Arenas[1], Elder Pupo[2], Peter van der Ley[2], Jan Tommassen[1]

[1]*Utrecht University, Microbiology, Utrecht, Netherlands*
[2]*Institute for Translational Vaccinology, Bilthoven, Netherlands*

Background: *Bordetella pertussis* is the causative agent of whooping cough. A whole-cell vaccine was introduced in the fifties of the previous century but it was too reactogenic. Therefore, it was replaced by a subunit vaccine, which is, however, insufficiently effective.

Objectives: Our goal is to develop a new, effective, whole-cell *B. pertussis* vaccine. To this end, reactogenicity, which is caused by the lipid A moiety of the lipopolysaccharides, should be reduced.

Methods: The reactogenicity of lipid A is determined by the number and the length of the acyl chains and the number of phosphate groups. The length of the acyl chains is species specific and is generally determined by molecular rulers in the acyltransferases that attach these chains to the lipid A precursors during biosynthesis. We genetically expressed the acyltransferases LpxA, LpxD, and LpxL from other bacterial species in *B. pertussis*. Changes in lipid A structure were determined by mass spectrometry, and reactogenicity was determined *in vitro* using HEK-Blue reporter cells expressing human TLR4.

Results: Heterologous expression of acyltransferases in *B. pertussis* resulted in new lipid A species with altered acyl-chain length. Shorter acyl chains at all positions resulted in reduced reactogenicity in TLR4 stimulation assays, whereas longer acyl chains enhanced reactogenicity. We also found that the unusual asymmetry of *B. pertussis* lipid A with respect to the acyl-chain length at the 3 and 3' positions is determined by substrate specificity of LpxH. In conclusion, lipid A engineering can be used to generate new, safe, whole-cell pertussis vaccines.

O258 High-affinity chemotaxis to histamine of the human pathogen Pseudomonas aeruginosa

Andrés Corral-Lugo[1], Miguel A. Matilla[2], David Martín-Mora[2], Hortencia Silva Jiménez[3], Noel Mesa Torres[2], Junichi Kato[4], Akiko Hida[4], Shota Oku[4], Mayte Conejero-Muriel[5], Jose Antonio Gavira[5], Tino Krell[2]

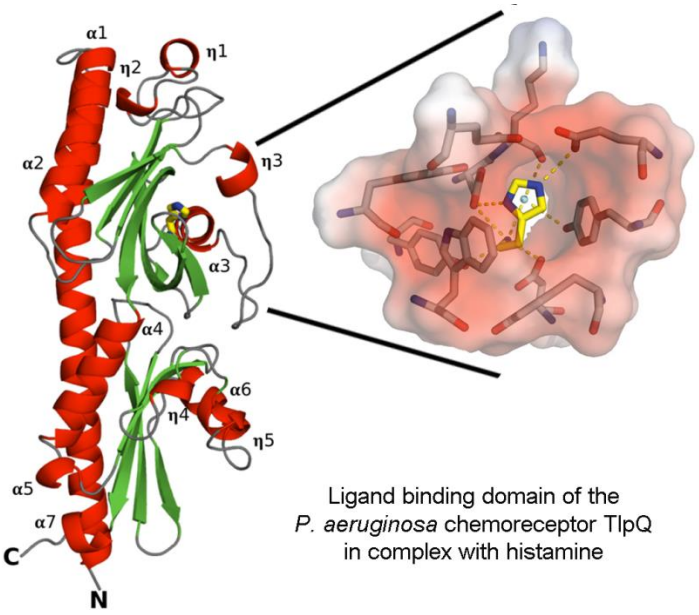
[1]*Institute Of Biology Intégrative De La Cellule Institute Of Biology Intégrative De La Cellule, Gif-sur-Yvette, France*
[2]*Zaidín Experimental Station, Granada, Spain*
[3]*INSTITUTO DE INVESTIGACIONES OCEANOLOGICAS, Ensenada, Mexico*
[4]*Hiroshima University, Higashihiroshima-shi, Japan*
[5]*Instituto Andaluz de Ciencias de la Tierra, Armilla, Spain*

Background: Chemotaxis allows bacteria to access nutrients as well as to colonize hosts and specific environmental niches. *Pseudomonas aeruginosa* PAO1 has 26 chemoreceptors of which most are of unknown function. In this pathogen, chemotaxis was shown to be necessary for optimal virulence.

Objectives: We aimed at identifying ligands that bind to *P. aeruginosa* chemoreceptors and to determine the funcion of these chemoreceptors.

Methods: High-throughput ligand screening, isothermal titration calorimetry, quantitative capillary chemotaxis assays, X-ray crystallography.

Results: PAO1 was found to show strong chemotactic responses to histamine over a concentration range of 500 nM to 50 mM. This response is due to the concerted action of the chemoreceptors PctA, PctC and TlpQ. TlpQ mediates chemotaxis to low histamine concentrations and its ligand binding domain (LBD) bound histamine with an unusually high affinity (K_D= 639 nM). The X-ray structure of TlpQ-LBD in complex with histamine reveals that bacterial histamine receptors are entirely different to their human counterparts. PctA and PctC respond to high histamine concentrations and are likely to be stimulated by the binding of histamine-loaded binding proteins. Histamine chemotaxis may result in an increase in virulence of *P. aeruginosa* since this pathogen was shown to greatly increase neutrophil histamine content and secretion in mouse models. Therefore, chemotaxis to this host-derived signal will result in an accumulation of bacterial cells at the infection site and this increase in cell density will likely alter the expression of quorum-sensing controlled genes, including those responsible for the production of virulence determinants.



Ligand binding domain of the
P. aeruginosa chemoreceptor TlpQ
in complex with histamine

O259 Signal transmission from the outer membrane to the cytosol through bacterial cell-surface signalling

Sarah Wettstadt[1], Joaquín Otero-Asman[1], Cristina Civantos[1], Elena Valdés[1], María Llamas[1]

[1]*Estación Experimental del Zaidín-CSIC, Environmental Protection, Granada, Spain*

Background: Many bacterial adaptations to changing environments are regulated by a signal transduction mechanism known as cell-surface signaling (CSS). In this regulatory cascade an environmental signal activates an outer membrane receptor and is further transmitted to an anti-sigma factor in the inner cell membrane. Signal transmission results in anti-sigma factor proteolysis and release of a sequestered extracytoplasmic function (ECF) sigma factor in the cytosol and thus gene transcription.

Objectives: We aimed at unravelling the molecular mechanisms behind signal transmission of the *Pseudomonas aeruginosa* ferrioxamine-induced Fox system. Specifically we focussed on the periplasmic interactions between the receptor FoxA and its corresponding anti-sigma factor FoxR and on how this interaction modulates the cognate ECF sigma factor (FoxI) activity.

Methods: We studied FoxA-FoxR interactions using isothermal titration calorimetry, bacterial two hybrid assays and *in-vivo* cross linking. Protein processing and stability was detected by western blot using specific antibodies. The physiological impact of FoxA-FoxR interactions was assayed *in vivo* by measuring FoxI sigma activity using a FoxI-dependent *lacZ* reporter construct.

Results: We previously showed that the periplasmic domain of FoxR is cleaved prior to signal recognition producing two domains (N- and C-domains). In this work we show that these two domains interact and that FoxA interacts solely with the FoxR C-domain. By introducing point mutations and deletions we could map the interaction surface and the impact of this interaction on FoxR proteolysis and FoxI activity. A new model for signal transmission by CSS linking FoxA-FoxR interaction with FoxR proteolysis and FoxI activity is proposed.

O260 pf4 phages production and/or infection induced different cell envelope stress responses in pseudomonas aeruginosa

Damien Tortuel[1], Ali Tahrioui[1], Sophie Rodrigues[1], Olivier Maillot[1], Onyedikachi Azuama[1], Olivier Lesouhaitier[1], Thomas Clamens[1], Laure Taupin[2], Alain Dufour[2], Emile Béré[3], Julien Verdon[4], Marc Feuilloley[1], Nicole Orange[1], Pierre Cornelis[1], Sylvie Chevalier[1], Emeline Bouffartigues[1]

[1]Laboratory of Microbiology Signals and Microenvironment - LMSM EA4312, Évreux, France
[2]Laboratory of Marine Biotechnology and Chemistry - LBCM - EA3884, IUEM, Lorient, France
[3] Image UP, Service de Microscopie Electronique et Photonique, Université de Poitiers, Campus Sciences, Pôle Biologie Santé, Bâtiment B36, Poitiers, France
[4]Laboratoire Ecologie & Biologie des Interactions, UMR CNRS 7267, Université de Poitiers, Poitiers, France

Background: Cell envelope stress responses (CESR) detect cell wall integrity alterations and reprogram gene expression to ensure bacterial survival. The underlying signal transduction is mediated partly by extracytoplasmic function (ECF) σ factors. *Pseudomonas aeruginosa* is a human opportunistic pathogen causing acute and chronic infections, which displays two major CESR systems based on the two ECF σ factors AlgU and SigX. In addition to controlling and maintaining peptidoglycan and membrane homeostasis, AlgU and SigX are master regulators involved in motility, biofilm formation and virulence in *P. aeruginosa*.

Objectives: Since phage infection and/or production require envelope crossing, we suspected CESR to be activated.

Methods: Pf4 filamentous phages addition led to a weak *P. aeruginosa* cell lysis and abrogated twitching motility. Expression and activity of AlgU and SigX were hugely increased in response to Pf4 infection, suggesting that exogenous Pf4 are perceived as major envelope stress signals. In addition, a mutant in which a Mini-*Himar1* transposon was inserted into the Pf4 prophage genomic island, produced and released constitutively phage particles, leading to a weak cell lysis, reduced twitching motility and adhesion, and altered biofilm architecture containing stretched cells. Remarkably, only AlgU, but not SigX, expression and activity were increased in the mutant strain, but too much lesser extent compared to treatment of *P. aeruginosa* with exogenous Pf4.

Results: Taken together, we show Pf4, CESR, motility and biofilm are connected at least through 2 different pathways involving AlgU and SigX in response to a “phage shock”, or only AlgU when Pf4 is released constitutively.

O261 A prophage encoded enzyme alters cell wall glycosylation in MRSA to evade immunity

David Gerlach [2], Yinglann Guo[3], Cristina De Castro[4], Sun-Hwa Kim[5], Katja Schlatterer[2], Fei-Fei Xu[6], Claney Pereira[6], Peter H. Seeberger[6], Sara Ali[7], Jeroen Codée[7], Wanchat Sirisarn[1], Berit Schulte[8], Christiane Wolz[8], Antonio Molinaro[9], Jesper Larsen[10], Bok Luel Lee[5], Guoqing Xia[1], Thilo Stehle[3], Andreas Peschel[2]

[1]*Lydia Becker Institute of Immunology and Inflammation, Division of Infection, Immunity and Respiratory Medicine, Faculty of Biology, Medicine and Health, University of Manchester*
[2]*Interfaculty Institute of Microbiology and Infection Medicine, Infection Biology, University of Tübingen*
[3]*Interfaculty Institute of Biochemistry, University of Tübingen*
[4]*Department of Agricultural Sciences, University of Naples, Naples, Italy*
[5]*National Research Laboratory of Defense Proteins, College of Pharmacy, Pusan National University, Pusan, South Korea*
[6]*Max-Planck-Institute for Colloids and Interfaces, Potsdam, Germany*
[7]*Leiden Institute of Chemistry, Leiden University, Leiden, The Netherlands*
[8]*Interfaculty Institute of Microbiology and Infection Medicine, Medical Microbiology, University of Tübingen*
[9]*Department of Chemical Sciences, University of Naples, Naples, Italy*
[10]*Bacteria, Parasites and Fungi, Statens Serum Institut, Copenhagen, Denmark*

Background: Methicillin-resistant Staphylococcus aureus (MRSA) is a frequent cause of difficult-to-treat, often fatal infections in humans . Previous vaccine development programs agaist S aureus have not been successful. A large percentage of human antibodies against S. aureus target wall teichoic acid (WTA), a ribitol-phosphate (RboP) surface polymer modified with N-acetylglucosamine (GlcNAc).

Objectives: We aim to study if TarP , a prophage encoded glycostransferase canmodify wall teichoic cids and alter host immune response

Methods: Bacterial genetics, glycochemistry, protein crystallography , immunology

Results: Here we show TarP can transfers GlcNAc to a different hydroxyl group of the WTA RboP than the standard enzyme TarS. TarP-glycosylated WTA elicits 7.5–40-fold lower levels of immunoglobulin G in mice than TarS-modified WTA. Consistent with this, human sera contained only low levels of antibodies against TarP-modified WTA. Notably, mice immunized with TarS-modified WTA were not protected against infection with tarP-expressing MRSA, indicating that TarP is crucial for the capacity of S. aureus to evade host defences. Structural analyses of TarP with WTA oligos and UDP-GlcNAc explain the mechanism of altered RboP glycosylation and form a template for targeted inhibition of TarP.

Our study reveals an immune evasion strategy of S. aureus based on averting the immunogenicity of its dominant glycoantigen WTA. These results will help design S. aureus vaccine antigens and may enable the development of TarP inhibitors as a new strategy for rendering MRSA susceptible to human host defences.

Reference: (1) Gelach, D, Guo, Y , et al, 2018, Nature, 563, 7733, 705-709.

O262 Insights into the recycling of the bacterial undecaprenyl-phosphate, the essential peptidoglycan carrier lipid

Meriem El Ghachi [1], Nicole Howe[2], Xudong Tian[3], Rodolphe Auger[3], Dominique Mengin-Lecreulx[3], Frédéric Kerff[1], Martin Caffrey[2], Thierry Touzé[3]

[1]*Centre d'Ingénierie des Protéines, Liège, Belgium*
[2]*Trinity College Dublin, School of Biochemistry and Immunology and Shool of Medicine, Dublin, Ireland*
[3]*Institute for Integrative Biology of the Cell, Microbiology, Orsay, France*

Background: The biogenesis of the bacterial cell-wall peptidoglycan implies the shuttle of glycan subunits across the plasma membrane. Individual building blocks are attached to the undecaprenyl-phosphate (C_{55}-P) at the cytoplasmic side of the membrane before being flipped to the periplasm, where they are transferred to the growing polymer releasing undecaprenyl-pyrophosphate (C_{55}-PP). C_{55}-P being present in a low number of copy per cell; it must be rapidly regenerated from this released C_{55}-PP and flipped back to the membrane inner side to fuel the high rate of peptidoglycan biosynthesis.

Objectives: We revealed in *Escherichia coli* the existence of a plurality of integral membrane phosphatases belonging to two unrelated families of proteins capable of converting C_{55}-PP in C_{55}-P. Our goal was to further determine whether this redundancy was shared in the bacterial world and to highlight its physiological relevance.

Methods: We used complementary appraoches ranging from genetic and phenotypic analyses to assert the role of different putative C_{55}-PP phosphatases belonging to the PAP2 (phosphatidic acid phosphatases of type 2) and the BacA families to their biochemical and structural characterization.

Results: Specific panels of C_{55}-PP phosphatases were determined in different bacteria. Dual functions of PAP2 enzymes were highlighted with some being involved in phospholipids or lipopolysaccharides biosynthesis in addition to C_{55}-P metabolism. The structure of one member of each family was obtained allowing further mechanistic investigations. Strikingly, the topology of BacA raised the hypothesis that BacA could also function as a flippase allowing the relocalisation of C_{55}-P product back to the membrane inner side.

O263 Structural Characterization of FmtA, the Modulator of Teichoic Acids of Staphylococcus aureus

Dasantila Golemi-Kotra[1], Vikram Dalal[2], Pravindra Kumar[2]

[1]Toronto, Toronto, Canada
[2]Roorkee, Roorkee, India

Background: FmtA is a D-amino esterase that removes the D-Ala group of teichoic acids. Wall teichoic acids are polymers involved in many physiological aspects of S. aureus, among them the recognition and infection by S. aureus specific phages. FmtA is similar to a penicillin-binding protein but it is devoid of such function. This protein is involved in biofilm formation and its knockout re-sensitizes MRSA to beta-lactams.

Objectives: We have undertaken structural biology and kinetic studies to reveal the structural elements that enable the D-amino esterase acticity of FmtA.

Methods: X-ray crystallography was used to determine the crystal structure of FmtA.

Results: 3D structure of FmtAresembles largely that of DD-carboxypeptidase enzymes and Class C beta-Lactamases. However, Local structural changes to the active site of the enzyme are unique to FmtA and hold the key to the substrate specificity of FmtA. The structure of FmtA offers an excellent target to understand the structural elements that drive substrate specificity among penicillin recognition proteins and their diversity in function. The crystal structure provides insights into targeting FmtA with the purpose of increasing S. aureus sensitivity to beta-lactam antibiotics and inhibiting biofilm formation.

O264 Comparison of enzymatic activity and oligomer formation in type Vd-secreted phospholipases

Thomas Trunk[1], Michael Casasanta[2], Christopher Yoo[2], Daniel Slade[2], Jack Leo[1]

[1]*University of Oslo, Department of Biosciences, Oslo, Norway*
[2]*Virginia Polytechnic Institute and State University, Department of Biochemistry, Blacksburg, United States*

Background: Type V secretion systems (T5SSs) or autotransporters as well as bacterial phospholipases are known virulence factors in many pathogenic bacteria. The patatin-like protein D (PlpD) of *Pseudomonas aeruginosa* is the prototype of the subclass T5dSS, which secretes a lipolytic passenger that forms extracellular homodimers.

Objectives: We aimed to characterize the enzymatic activity, substrate specificity and multimerization status of homologues of PlpD from the pathogens *Aeromonas hydrophila*, *Burkholderia pseudomallei*, *Ralstonia solanacearum* and *Vibrio cholerae*.

Methods: The lipase domains of T5dSSs were produced recombinantly. A continuous fluorimetric assay was used to measure lipase activity of the target proteins. Substrate specificity was assayed using lipid strips, and oligomer formation was determined by size-exclusion chromatography and crosslinking.

Results: All studied phospholipases were active over a broad temperature and pH range, displayed phospholipase A1 (PLA1) activity and high-affinity binding to phosphatidylserine. Phospholipases from *A. hydrophila* and *B. pseudomallei* also showed PLA2 activity. Based on size exclusion chromatography and crosslinking, self-associated multimer formation seems to be a conserved feature of T5dSS passengers. Yet, homodimer formation is not essential for enzymatic activity but rather for protein stability. The exact role that T5dSSs play during infection has yet to be determined, but the fact that several of the characteristics tested in this work are conserved across a wide range of pathogens and that there are distinctive correlations between enzymatic activity as well as substrate specificity and intra- and extracellular lifestyle suggests an important role for T5dSS phospholipases for bacterial fitness.

O265 Targeting the Fungal Cell Wall

Carol Munro[1]

[1]*University of Aberdeen, MRC Centre for Medical Mycology, Aberdeen, United Kingdom*

Background: The fungal cell wall is a dynamic organelle composed of chitin, β-1,3-glucan, β-1,6-glucan and mannoproteins. The polysaccharides give the wall its mechanical strength and the mannoproteins include important virulence factors as well as carbohydrate active enzymes that contribute to mainitaing cellular integrity. Fungi can alter the composition, structure and properties of their outer coats in response to different internal and external stimuli such as mutations in cell wall associated genes and changes in environmental conditions.

Objectives: To understand the impact of cell wall remodelling on drug susceptibility, host interactions and fungal pathogenesis. To compare the cell wall of drug sensitive and drug resistant clinical isolates to gain a better understanding of the role of the cell wall in drug tolerance and resistance mechanisms.

Methods: We are investigating the fungal cell wall using a combination of carbohydrate biochemistry, proteomics, fluorescence and electron microscopy. We study the impact of genetic changes on wall and virulence associated phenotypes. The genetic changes may be due to targeted gene deletion or gene overexpression in mutants we engineer in the laboratory as well as acquired mutations in response to exposure to antifungal drugs in patients with invasive fungal infections.

Results: Our analyses have highlighted changes in cell wall composition such as elevated chitin levels and the increased expression of cell surface proteins in response to antifungal drug treatment and in drug resistant isolates. We have also characterised pathogen-specific cell surface protein families that have roles in adhesion, biofilm formation, interactions with the host and pathogenicity.

O266 The Cryptococcus neoformans TItan cell is an inducible and regulated morphotype underlying pathogenesis

Xin Zhou[1], Raif Yeucel[2], Donna MacCallum[2], Ivy Dambuza[2], Elizabeth Ballou[1]

[1]*University of Birmingham, Institute of Microbiology and Infection, Edgbaston, United Kingdom*
[2]*University of Aberdeen, Institute of Medical Sciences, Aberdeen, United Kingdom*

Background: Fungal cells change shape in response to environmental stimuli, and these morphogenic transitions drive pathogenesis and niche adaptation. For example, dimorphic fungi switch between yeast and hyphae in response to changing temperature. The basidiomycete *Cryptococcus neoformans* undergoes an unusual morphogenetic transition in the lung from haploid yeast to large, highly polyploid cells termed Titans. Titans influence fungal interaction with host cells, including through increased drug resistance, altered cell size, and altered Pathogen Associated Molecular Pattern exposure.

Objectives: Despite the important role these cells play in pathogenesis, understanding the environmental stimuli that drive the morphological transition, and the molecular mechanisms underlying their unique biology, has been hampered by the lack of a reproducible *in vitro* induction system.

Methods: We recently demonstrated that Titan cells can be induced *in vitro* in response to environmental stimuli consistent with the host lung

Results: . Significantly, we showed that bacterial cell wall serves as a key component in this process. *In vitro* Titans exhibit all the properties of *in vivo* Titans, including altered capsule, cell wall, size, high mother cell ploidy, and aneuploid progeny. Using this model, we begin to describe molecular mechanisms underlying the yeast-to-Titan transition, investigate Titanisation in clinical isolates, and characterize their impact on host interaction and disease outcome.

O267 Functional genomics of biofilm formation by Candida albicans

Christophe D'Enfert[1], El Ghalid Mennat[1], Rai Laxmi[1], Bachellier-Bassi Sophie[1]

[1]*Institut Pasteur, Fungal Biology and Pathogenicity, Paris, France*

Background: Infections due to the yeast *Candida albicans* have emerged over the past three decades as a considerable threat to human health. *C. albicans* forms therapeutically-challenging biofilms on biomedical implants which exhibit elevated intrinsic resistance to several antifungal agents, in particular azoles and polyenes.

Objectives: We aim to elucidate the molecular mechanisms involved in the formation and antifungal tolerance of biofilms as their knowledge is likely to yield novel strategies for combatting biofilm-associated infections.

Methods: Our approach for the identification of *C. albicans* genes involved in biofilm formation leverages a genome-wide collection of *C. albicans* signature-tagged overexpression strains that can be evaluated individually or in pools for their ability to form biofilms. Further characterization of the genes whose overexpression positively or negatively impacts biofilm formation involves functional genomics approaches as well as structural biology of cell surface proteins.

Results: Characterization of > 2500 *C. albicans* overexpression strains for their ability to form biofilms on abiotic surfaces has now revealed a number of genes that contribute to biofilm formation in this species. In particular, a signature-tagged mutagenesis approach aimed at identifying genes whose overexpression impacts the ability of *C. albicans* to thrive within biofilms has been used and has revealed that a number of so far uncharacterized cell surface proteins can contribute to biofilm formation through cell-to-surface or cell-to-cell interactions. Further characterization of these cell wall proteins using structural biology approaches suggest that some can form amyloids that contribute to adhesion to cell surfaces and biofilm formation.

O268 Carbon metabolism coordinates virulence and immunogenicity in the fungal pathogen Candida albicans

Slavena Vylkova[1;2;3], Enrico Gerbe[1;2;3], Bettina Boettcher[1;2;3], Robert Williams[4], Michael Lorenz[4]

[1]*Hans-Knöll Institute*
[2]*Friedrich-Schiiler University*
[3]*Septomics Research Center*
[4]*University of Texas Health Science Center in Houston, Houston, United States*

Background: *Candida albicans* is a commensal fungal microorganism in humans that can become pathogenic and infect virtually any part of the body. Within the host *C. albicans* can utilize a variety of non-preferred carbon sources, including amino acids, carboxylic acids, or N-acetylglucoasmine. Though the routes of catabolism of these nutrients are independent, all three share a common feature: they allow *C. albicans* to neutralize initially acidic environments, including the macrophage phagolysosome.

Objectives: To examine the role of *C. albicans* mutants that fail to neutralize the environment in virulence and immunogenicity.

Methods: *C. albicans* mutants lacking the regulator of amino acid permeases Stp2, carboxylic acid permeases Jen1 and Jen2, or the genes involved in GlcNAc catabolism were tested for environmental neutralization and morphogenesis *in vitro* and within phagolysosomes. *stp2Δ* cells were tested for adherence, biofilm formation and virulence.

Results: All mutants failed to induce morphogenesis, occupy more acidic phagolysosomes and were readily killed by macrophages compared to the controls. Transcriptional profiling revealed that aside from amino acid regulation, Stp2 controls the expression of hyphae-associated genes, including *ECE1* and *HWP1*, suggesting novel function for this transcription factor. Indeed, *stp2Δ* cells showed delayed hyphae formation in hyphae-inducing conditions, while *STP2* overexpression resulted in more robust morphogenesis. Further, *stp2Δ* showed reduced adherence to abiotic and biotic surfaces, and biofilm formation under static and sheer flow conditions. Stp2 was also required for full virulence in systemic model of candidiasis. In summary, *C. albicans* catabolism of non-preferred carbon sources has profound effects on host pathogen interaction.

O269 From commensalism to pathogenicity: interactions of Candida albicans with the host

Bernhard Hube[1]

[1]*Leibniz-Institute for Natural Product Research and Infection Biology - Hans Knöll Institute, Department Microbial Pathogenicity Mechanisms, Jena, Germany*

Background: The fungus *Candida albicans* is both, a commensal and an opportunistic pathogen. The adaptation of this fungus to the human host is the result of an ancient, mostly commensal, relationship and has led to the development of distinct fungal strategies to survive and proliferate in diverse host niches.

Objectives: For its transition to a pathogenic phase in the host, it relies on attachment to, invasion into, and damage of epithelial cells. Adhesion is mediated by fungal surface proteins and stimulates hyphae production and expression of hypha-associated genes. These filaments are not only more adhesive, but also more invasive than yeast cells. Invasion is accompanied by epithelial damage, however, it does not necessarily cause damage *per se*. In fact, it has become clear that most of the damage is due to a hypha-associated polyprotein containing the first peptide toxin identified in a human pathogenic fungus.

On the other hand, the host has adapted and evolved mechanisms to prevent invasion and infection by any type of microbes, including *C. albicans*. This co-evolution scenario has not only led to the emergence of distinct microbial virulence factors, but also to “avirulence factors” (well known in the plant pathology field) or “immune modulators” (in the field of human immunology), which, once expressed by the microbial pathogen, are recognized by the host and trigger microbial clearance *via* immune responses.

Results: In *C. albicans*, we found examples of proteins which contribute to both offence and defence, and which are thus both virulence and avirulence factors.

O270 Epigenetic control of lineage formation by DNA adenine methylation

Josep Casadesus[1]

[1]*Universidad de Sevilla, Departamento de Genética, Sevilla, Spain*

In gamma-Proteobacteria, formation of DNA adenine methylation patterns (combinations of methylated and nonmethylated GATC sites) provides a mechanism for reversible switching of gene expression, and for the propagation of epigenetic states to daughter cells. Nonmethylated GATC sites are often part of clusters of 2-4 GATCs located within binding sites for transcriptional regulators, and are flanked by DNA sequences that reduce the processivity of Dam methylase. Binding of the cognate protein hinders Dam methylase activity, generating nonmethylated GATCs after two rounds of DNA methylation. Nonmethylation persists as long as the transcription factor remains bound to its cognate sequence, and is heritable. However, every DNA replication round provides a window of opportunity to change the DNA methylation pattern of the regulatory region, switching transcription from OFF to ON and *vice versa*. A paradigm of Dam-dependent epigenetic control is the *pap* operon of uropathogenic *E. coli*, whose workings were unraveled by David Low and co-workers in the 1990s. In *pap*, transcriptional switching is controlled by the methylation state of 2 GATCs, and the transcription factor involved is Lrp. Additional examples of loci harboring Dam-dependent switches are *agn43* and *sciH* in *E. coli*, and *gtr*, *opvAB*, and *std* in *Salmonella*. In *agn43*, *gtr*, and *opvAB*, transcriptional switching is under OxyR control. Switching of *std* is controlled by a logic AND-gate involving HdfR, a poorly known transcriptional regulator, and *std*-encoded proteins StdE and StdF. The adaptive value of Dam-dependent lineage formation can be understood in certain cases, and will be discussed.

O271 Listeria impact on host epigenetics

Matthew Eldridge[1], Pascale Cossart[1]

[1]*Institut Pasteur, Cell Biology and Infection, Paris, France*

The field of epigenetics has received a lot of attention recently in particular during infection. Indeed, pathogens for efficient infection reprogram the host transcription. They do so by various means that affect the chromatin itself or chromatin factors. One of these means is via nucleomodulins, i.e. bacterial proteins produced by the pathogens, that enter the nucleus and act in various ways. In *Listeria* for example, a small protein called LntA interacts with and desequesters a previously unknown heterochromatinization factor called BAHD1 allowing expression of a number of genes critical for infection. Another means is via histone modifications and we have shown that *Listeria* induces a deacetylation of histone H3 via Sirtuin2, a deacetylase previously reported to act preferentially on cytoplasmic substrates such as tubulin during interphase. Deacetylation of H3 leads to gene repression, an event critical for efficient infection. We have shown that Sirtuin2 translocates to the nucleus and associates with chromatin if and only if Serine 25 is dephosphorylated, an event mediated by PPM1A and PPM1B translocation to the nucleus.

O272 Causes and consequences of phenotypic heterogeneity in Salmonella virulence gene expression

Martin Ackermann[1]

[1]*ETH Zurich and Eawag, Department of Environmental Systems Sciences, Zurich, Switzerland*

Background: Individual bacterial cells in clonal populations often exhibit a marked degree of phenotypic heterogeneity: cells differ with respect to gene expression, metabolism and behavior. Phenotypic heterogeneity allows clonal populations of pathogens to evade host responses, survive treatment with antibiotics, and engage in the division of labor during the colonization of host compartments. While many studies support the diverse roles of phenotypic heterogeneity for bacterial infections, some important questions remain largely unresolved: we often do not know the molecular mechanism that produce phenotypic differences between genetically identical cells; we do not know how the degree of phenotypic heterogeneity is modulated by external factors; and we often do not understand how exactly phenotypic heterogeneity allows pathogens to establish and maintain infections.

Objectives: Working with the bacterial pathogen *Salmonella* Typhimurium, our goal is to work on these knowledge gaps on the causes and consequences of phenotypic heterogeneity.

Methods: We use quantitative single-cell measurements to analyze the temporal dynamics of virulence gene expression in individual *Salmonella* cells and the modulation of phenotypic heterogeneity by external factors.

Results: Quantitative single-cell measurements of *Salmonella* genes that are involved in virulence and host interaction reveal oscillations in the expression of some of these genes and show that size and behavior of phenotypic sub-populations are modulated by external factors, and potentially controlled by other members of the gut microbiota. I will discuss whether some of these findings can be translated to other pathogens, and potentially lead the way to new approaches for infection control.

O273 Salmonella persisters undermine host immune defences during antibiotic treatment

Sophie Helaine[1], Daphne Stapels[1]

[1]*Imperial College London, MRC CMBI, London, United Kingdom*

Background: Many bacterial infections are hard to treat and tend to relapse, due to the presence of persister cells. The internalisation of *Salmonella* by macrophages induces formation of such non-growing persisters that, in contrast to bacterial persisters formed in laboratory medium, remain in a metabolically-active state.

Objectives: Here we investigate how the metabolic activity of persisters supports their survival during extended periods of time in the host.

O274 Contribution of new developments in the synthetic biology toolbox to the engineering of yeast cell factories

Pascale Daran-Lapujade[1]

[1]*Delft University of Technology, Department of Biotechnology, Industrial Microbiology section, Netherlands*

The push towards sustainable alternatives to oil-derived chemicals requires the construction of powerful microbial cell factories that can produce new chemicals, using unnatural substrates at high yields and rates, under harsh industrial conditions. Irrespective of the type of product, optimal performance of engineered cells requires modifications in the activity and regulation of core cellular and metabolic functions in the host organism. Achieving optimal microbial performance in industrial settings is far from trivial because it typically conflicts with the natural propensity of microbes to prioritize resource utilization for growth and survival. The construction of powerful cell factories therefore demands extensive genetic remodeling of the host microbes. Rapid developments in the field of synthetic biology over the past few years have delivered new molecular tools that strongly enhance the genetic accessibility of microbial genomes. Using the model and industrial yeast *Saccharomyces cerevisiae* as paradigm, we will explore how these synthetic tools have empowered the construction of yeast cell factories and will reflect on the remaining challenges.

O275 Rewiring yeast central carbon metabolism for industrial isoprenoid production

Annie Tsong[1]

[1]*Amyris, Research and Development, Emeryville, United States*

Background: A bio-based economy has the potential to provide sustainably-derived substitutes for petroleum-based products, as well as new chemical building blocks for advanced materials. At Amyris, we have engineered the yeast *Saccharomyces cerevisiae* for commercial production of isoprenoid-based pharmaceuticals and specialty chemicals. However, the native central metabolism of *Saccharomyces cerevisiae* is chemically suboptimal in conversion of feedstock to isoprenoids, which prevents economically viable production at the commodity-scale.

Objectives: We set out to rewire the central carbon metabolism of *S. cerevisiae* to enable production of isoprenoids at volumes and costs which can compete with commodity-scale petrochemicals.

Methods: Using computational modeling, we identify four non-native metabolic reactions which enable biosynthesis of cytosolic Acetyl Coenzyme A (the two carbon isoprenoid precursor) with reduced ATP requirement, reduced loss of carbon to CO2-emitting reactions, and improved pathway redox balance. We then express enzymes that catalyze these four reactions in an industrial isoprenoid-producing strain background, and eliminate competing native reactions.

Results: We demonstrate that that strains with rewired central metabolism devote an identical percentage of sugar consumed to farnesene production as control strains using the native central metabolism, yet produce 25% more farnesene with that sugar while requiring 75% less oxygen – precisely in line with computational predictions. These changes lower feedstock costs and dramatically improve capital utilization of industrial fermenters which are by necessity oxygen-contrained. This illustrates that rewiring yeast central metabolism is a viable strategy for cost-effective, large-scale production of Acetyl Coenzyme A-derived molecules.

O276 Optimising synthetic biology by working with the host cell

Tom Ellis[1]

[1]*Imperial College London, United Kingdom*

Synthetic DNA constructs can be used to reprogram cells to sense, compute, record information and to produce chemicals, medicines and materials. However, the functions of these constructs can impact on their host cell, and conversely the host cell background can also impact on the efficiency of the construct function. To uncover how construct function impacts on a host, we used multiplex RNAseq with an *in vivo* assay to reveal the major transcriptional changes that occur in response to synthetic construct expression. Our systems analysis led us to identify native biosensors for unnatural expression in *E. coli* strains and exploit these to build a CRISPR/dCas9-based feedback regulation system that automatically adjusts expression to ensure robust growth in many varied conditions. In parallel, to investigate how host background impacts a construct, we assessed the performance of metabolic pathway-encoding constructs hosted in synthetic yeast strains that have chromosomes that can be rearranged on demand. *In vivo* deletion and inversion of chromosomal regions quickly enabled new synthetic yeast host cells to be generated with greatly-enhanced xylose utilisation, and improved violacein and penicillin biosynthesis. We are now exploring how host cell genomes can be engineered to make strains specific for different tasks, including biosensing.

O277 Creating synthetic yeast cell factories for oleochemicals and isoprenoids

Irina Borodina[1]

[1]*Technical University of Denmark, The Novo Nordisk Foundation Center for Biosustainability, Kgs. Lyngby, Denmark*

Background: Oleaginous yeast *Yarrowia lipolytica* possesses a remarkable ability to accumulate high levels of lipids and to secrete organic acids. With the recent advances in CRISPR-based genome editing, this yeast has become well amenable to genome editing.

Objectives: We aimed to engineer *Y. lipolytica* into a platform cell factory, well suitable for production of oleochemicals and isoprenoids.

Methods: We introduced biosynthetic routes towards high-value specialty chemicals, carotenoids, lactones, and insect pheromones, into *Y. lipolytica*. Furthermore, we engineered the metabolism of the yeast to redirect a larger carbon flux into the products.

Results: The engineered strains produced high levels of astaxanthin, canthaxanthin, lactone flavours, or insect pheromones. This enables the replacement of the current production routes of these compounds with fermentation, resulting in product cost reduction and environmental benefits. Carotenoid-type pigments astaxanthin and canthaxanthin are useful as fish and poultry feed additives. Lactone flavors are used in food and cosmetics. Insect pheromones are applied for environmentally friendly pest control.

O278 Phage-Bacteria Interaction in the Coastal Ocean

Martin Polz[1]

[1]*Massachusetts Institute of Technology, Civil and Environmental Engineering, Cambridge, United States*

Background: Viruses are key members of all ecosystems and exert top-down control of microbial populations. By selecting for resistance, viruses are thought to be major drivers of the diversity observed among closely related microorganisms. However, the eco-evolutionary dynamics of these interactions remain poorly understood.

Objectives: To determine the patterns and mechanisms of phage-host interactions in the coastal marine environment.

Methods: We used a combination of environmental sampling, virus-host infection determination, comparative genomics and molecular genetics.

Results: Analysis of the largest, fully genome-resolved cross-infection matrix of viruses and their bacterial hosts showed that viral predation is highly specific, differentiating nearly clonal isolates. This specificity is meadiate by mobile genetic elements that carry defense genes and displsy extremely rapid evolutionary turnover. Each bacterial strain can carry up to 5 different defense elements that, due to their considerable length, make up a sizeable portion of the pan-genome of populations. These defense islands are strikingly diverse, containing restriction-modification systems, recently discovered anti-phage genes, and additional unannotated loci shared among subsets of islands. Viral receptors at the cell surface of hosts, on the other hand, are suprisingly invariant and broadly distributed enabling diverse phage to inject their DNA into hosts where it is subsequently inactivated but available for genetic exchange. Hence phage have effectively broader host ranges than suggested from cross infection studies, explaining the paradox that phage are highly recombinogenic in spite of limited host range.

O279 Using experimental evolution to understand marine microbes in changing environments

Sinead Collins[1]

[1]*University of Edinburgh, Institute of Evolutionary Biology, United Kingdom*

Background: Marine microbes evolve on timescales of blooms, seasons, or a few years, and experimental evolution is one powerful tool for understanding how microbial populations can respond to environmental changes on various timescales

Objectives: Predicting trait changes in marine primary producers under ocean change

Methods: Experimental evolution, marine ecosystem modeling

Results: I will go over how "standard" evolution experiments designed to understand basic evolutionary processes, such as the role of epigenetic mutations or the role of environmental fluctuations in adaptation, can be used to improve models trait evolution in marine microbes under ocean warming.

O280 General and specific cross-feeding during marine phototroph-heterotroph interactions

Despoina Sousoni[1], Amandeep Kaur[1], David Scanlan[1], Joseph Alexander Christie-Oleza[1]

[1]*Unversity of Warwick, School of Life Sciences, Coventry, United Kingdom*

Background: Despite the scarcity of nutrients in the photic layer of the ocean, these prevail as highly productive systems that sustain the entire marine food web. The unicellular phototrophs that populate and feed these ecosystems have adapted to extreme oligotrophic conditions via various means. One of these is based on niche specialization which facilitates collaborative interactions, where nutrients are recycled between phototrophic and heterotrophic members of the planktonic community.

Objectives: To underpin the physiological phototroph-heterotroph interactions that take place under oligotrophic conditions.

Methods: We have performed long-term co-culture experiments in natural seawater using a broad range of model phototrophic microorganisms (i.e. picocyanobacteria *Prochlorococcus* and *Synechococcus*, green algae *Micromonas* and *Ostreococcus*, the haptophyte *Emiliania huxleyi*, and diatoms *Phaeodactylum tricornutum* and *Thalassiosira pseudonana*) grown in combination with a diverse range of marine heterotrophic bacteria.

Results: We show that it is not the concentration of nutrients but rather their circulation that maintains a stable interaction and a dynamic system, although this was not achieved under all combinations. High-throughput proteomic and metabolite data generated from these co-cultures revealed mechanistic insights into the cross-feeding process that occurs in each one of these systems and, thus, has provided a comprehensive understanding of the networks of marine phototroph-heterotroph interactions at a broad scale. Our results challenge the general belief that marine phototrophs and heterotrophs compete for the same scarce nutrients and niche space and, instead, suggest these organisms more likely benefit from each other because of their different levels of specialization and complementarity within long-term stable-state systems.

O281 Laminarin is a key molecule in the marine carbon cycle and fuels heterotrophic metabolism

Stefan Becker[1], Sarah Coffinet[1], Karen Wiltshire[2], Morten Iversen[1], Kai-Uwe Hinrichs[1], Jan-Hendrik Hehemann[3;4]

[1]*Marum University of Bremen, Bremen,*
[2]*Alfred Wegener Institute for Polar and Marine Research, Bremerhaven, Germany*
[3]*MARUM - Center for Marine Environmental Sciences University of Bremen, Bremen,*
[4]*Max Planck Institute for Marine Microbiology, Bremen,*

Background: Marine algae sequester as much CO_2 into carbohydrates as terrestrial plants. Polymeric carbohydrates, i.e., glycans provide carbon for heterotrophic bacteria and may form a potential sink of carbon in the ocean.

Objectives: Measure the quantitative contributions of marine algal glycans to establish their role as food for microbes for carbon cycling and for carbon sequestration.

Methods: We developed a biocatalytic strategy based on bacterial enzymes. The biocatalytic assay consists of bacterial laminarinases that cleave the algal glycan laminarin into readily analyzable fragments, which can be easily quantified with HPLC or with colorimetric assays.

Results: We measured laminarin concentrations in the Arctic, Atlantic, and Pacific oceans and during two North Sea time series and found that it accounts on average for 37±17% of the algal particulate organic carbon. An observed correlation between chlorophyll and laminarin suggests an annual algal laminarin production of 18±9 gigatons, that is, more than four times the annual atmospheric carbon dioxide increase by fossil fuel burning. Moreover, laminarin accounted for up to 50% of the carbon in sinking particles and thus contributed significantly to carbon export from surface waters. Spatially and temporally variable laminarin concentrations of up to 80% of the particulate organic carbon from the surface ocean were driven by light availability. These variations indicate the role of laminarin for carbon export and energy flow to bacteria as well as to higher trophic species and point to its still unconstrained role in marine ecosystems.

O282 Exploiting intracellular bacteria for medical biotechnology

Clare Taylor[1]

[1]*Edinburgh Napier University, School of Applied Sciences, Edinburgh, United Kingdom*

Background: Intracellular bacteria have evolved elegant mechanisms to invade, manipulate, and survive in their hosts. Investigation of bacterial pathogenic mechanisms has helped us to understand, in many cases, how intracellular bacteria overcome host immune responses but future developments include exploiting such bactreia for medical therapies. There is growing interest in the concept of 'anti-virulence' as an alternative therapeutic approach, particularly in the light of increasing antibiotic resistance. Furthermore, a number of approaches exploiting pathogenic mechanisms to deliver therapeutic molecules directly into the host have shown promise as anti-tumour therapies.

Objectives: Recent progress in exploiting bacteria such as *Salmonella enterica* and *Listeria monocytogenes* will be discussed, including some of the challenges and complexities that are known, as well as those which are as yet unknown.

O283 Exploring Predatory Bacteria as Living Antibacterials

Liz Sockett[1], Jess Tyson[1], Carey Lambert[1], Dhaarini Raghunathan[1], Paul Radford[1], Chris Moore[1], Michelle Baker[1;2], Ting Lai[1], Jamie Twycross[2], David Negus[1], Alex Willis[3], Serge Mostowy[3]

[1]*University of Nottingham, School of Life Sciences, Nottingham, United Kingdom*
[2]*University of Nottingham, Computer Sciences, Nottingham, United Kingdom*
[3]*Imperial College London, Centre for Molecular Bacteriology and Infection, London, United Kingdom*

Background: Gram negative E(S)KAPE Pathogens including E.coli, Klebsiella.pneumoniae, Acinetobacter baumanii show increasing resistance to conventional antibiotics and are listed by W.H.O. as requiring the urgent development of new counter-measures. We are researching the use of living predatory bacteria, such as *Bdellovibrio bacteriovorus* (small Gram-negative deltaproteobacteria that naturally invade and kill Gram-negative pathogens of humans, animals and plants) to treat such infections. As live, but non-pathogenic bacteria themselves, *B. bacteriovous* can evolve to adapt to different pathogens and they encode a large arsenal of antibacterial enzymes which they use in a programmed way in their predatory life cycle. As bacteria themselves, they bring potential issues of bodily spread and host immunity which must be explored

Objectives: Our goals are to evaluate *B. bacteriovorus* cells as live agents for bacterial killing and to test their interactions in live hosts and with host immune systems, to see how they may act *in vivo.*

Methods: We have previously applied whole *B. bacteriovorus* and shown that they combat Gram-negative pathogens in live poultry and food crops. Recently we have taken these studies further using a zebrafish hindbrain infection model and cultured human macrophage like U937 cells.

Results: We have shown that injection of *B. bacteriovorus* works alongside the immune system to clear otherwise lethal Gram-negative infections in zebrafish larvae and are now investigating how this dual effect can occur. We have also detected significant persistence of *B.bacteriovorus* in human macrophage like cells and will discuss the implications of these findings for future therapy.

O284 Optimising an anti-adhesion treatment for P. aeruginosa infections with the aid of mathematical modelling

Sara Jabbari[1], Paul Roberts[2], Emma Keen[3], Anne-Marie Krachler[4]

[1]University of Birmingham, School of Mathematics & Insitute of Microbiology and Infection, Birmingham, United Kingdom
[2]University of Birmingham, School of Mathematics & Institute of Microbiology and Infection, Birmingham, United Kingdom
[3]University of Birmingham, Institute of Microbiology and Infection, Birmingham, United Kingdom
[4]University Of Texas Health Science Center At Houston, Houston, United States

Background: The ability of bacteria to become resistant to previously successful antibiotic treatments is an urgent and increasing worldwide problem. Solutions can be sought via a number of methods including, for example, identifying novel antibiotics, re-engineering existing antibiotics or developing alternative treatment methods. The nonlinear interactions involved in infection and treatment render it difficult to predict the success of any of these approaches without the use of computational tools in addition to more traditional experimental work.

Objectives: We use mathematical modelling to aid in the development of anti-virulence treatments that, unlike conventional antibiotics that directly target a bacterium's survival, may instead attenuate bacteria and prevent them from being able to cause infection. Many of these anti-virulence treatments, however, are only partially successful when tested in infection models. We apply our interdisciplinary approach specifically to MAM7-based inhibitory beads, used to prevent *P. aeruginosa* from adhering to host cells (a key early stage in infection).

Methods: By carefully combining ordinary differential equation models with experimental data, we are able to produce reliable mathematical models of different treatment scenarios.

Results: Computational optimisation procedures are applied to make experimentally-testable predictions regarding combinations of treatments that should minimise the bacterial load. In particular, we consider an antibiotic-resistant infection and predict that MAM7-based inhibitory beads can be successful in clearing an antibiotic resistant infection when combined in an appropriate way with antibiotics and/or debridement.

O285 Engineering E. coli bacteria for the targeted injection of proteins into tumor cells

Luis Angel Fernandez[1]

[1]*Centro Nacional de Biotecnología (CNB-CSIC), Microbial Biotechnology, Madrid, Spain*

Background: One of the aims of synthetic biology is the design of microorganisms for the development of diagnostic sensors and therapeutic agents against human diseases such as cancer.

Objectives: Report the development of two important tools that enable to precisely program *E. coli* bacteria to: 1) adhere to specific target cells; and 2) assemble filamentous injectisomes from type III secretion systems (T3SS) that act as "molecular syringes" for translocation of specific proteins into mammalian cells.

Methods: We have generated synthetic adhesins (against different antigen targets expressed on the surface of mammaliam cells and have demonstrated the specific adhesion of the engineered *E. coli* bacteria to target tumor cells using *in vitro* and *in vivo* models. Secondly, we engineered the expression of functional injectisomes in a non-pathogenic commensal *E. coli* strain by reformatting the operons encoding the structural proteins and chaperones needed for the assembly of injectisomes from enteropathogenic *E. coli* (EPEC).

Results: The resulting strain, named Synthetic Injector E. coli (SIEC), was shown to translocate a natural EPEC effector called Tir into HeLa cells. Recent work has demonstrated that other effector proteins can be efficiently delivered by SIEC into mammalian cells. In addition, we have shown that SIEC can translocate heterologous proteins (e.g. antibody fragments) into human and mouse cell lines. In order to provide cell specificity for protein injection the expression of synthetic adhesins in SIEC bacteria. Our results show that SAs can be expressed in SIEC bacteria for the targeted delivery of a protein payload into tumor cells.

O286 Impact of genome diversity of Clostridium botulinum

Michael Peck[1], Jason Brunt[2], Sandra Stringer[1], Andrew Carter[1], Arnoud van Vliet[3]

[1]*Quadram Institute Bioscience, Norwich, United Kingdom*
[2]*University Of Cambridge, United Kingdom*
[3]*University Of Surrey, United Kingdom*

Background: *Clostridium botulinum* is a heterogeneous species comprising at least four distinct Groups of Gram-positive endospore-forming anaerobic bacteria. *C. botulinum* is responsible for botulism, a severe and deadly disease associated with consumption, or *in vivo* formation, of the highly potent botulinum neurotoxin. There are eight botulinum neurotoxin serotypes, and more than forty botulinum neurotoxin sub-types. There are three major types of human botulism; foodborne, infant and wound. Strains of *C. botulinum* Group I and Group II are responsible for most cases of human botulism. *C. botulinum* Group I is a mesophilic proteolytic bacterium that forms neurotoxin of serotypes A, B, F and/or X, and *C. sporogenes* is a close relative of *C. botulinum* Group I. *C. botulinum* Group II is a psychrotrophic saccharolytic bacterium that forms a single neurotoxin of serotype B, E or F.

Objectives: This study has been concerned with the analysis of genomes of strains of *C. botulinum* Group I and *C. botulinum* Group II, and has evaluated genome diversity in relation to the risk of botulism in humans.

Methods and Results: The increased availability of *C. botulinum* genome sequences has provided a better characterization of the genomic diversity of *C. botulinum* Groups I and II and their neurotoxins. Both *C. botulinum* Group I and *C. botulinum* Group II separate into a number of distinct sub-groups, and there are new insights into biology of these bacteria, their transmission, evolution, and the risk presented. This information will also enable improved surveillance and tracing/tracking during outbreaks.

O287 Clostridium perfringens strains causing foodborne illness

Matthew Callaghan[1], Kristian Kollerud[1], Jos Boekhorst[1], Marjon Wells-Bennik[1]

[1]*NIZO food research BV, Ede, Netherlands*

Background: The anaerobic sporeforming bacterium *Clostridium perfringens* is one of the most common causes of foodborne illness as a result of *C. perfringens* enterotoxin (CPE) production in the gut. Yet, only ~5% of strains carry the *cpe* gene, which can be located chromosomally (C-*cpe*) or on a plasmid (P-*cpe*). C-*cpe* strains have predominantly been associated with foodborne outbreaks as their spores may survive heating processes better than spores of P-*cpe*/non-*cpe* strains.

Objectives: To improve detection and gain insight in occurence in different environmental niches, genotypic and phenotypic characterization of *C. perfringens* outbreak strains (C-*cpe*/P-*cpe*) and non-*cpe* strains was performed with a focus on substrate utilization, growth properties and spore heat resistance.

Methods: Genome sequencing and analysis of 22 *C. perfringens* Type A strains (*C-cpe, P-cpe* and *cpe*-negative) showed that C-*cpe* strains and P-*cpe*/non-*cpe* strains clustered in separate phylogenetic groups. Unique genes and their predicted functions were identified. For each strain, spores were produced to asses heat inactivation kinetics, substrate utilization was tested using API, and growth was assessed at different temperatures.

Results: 152 genes were unique to strains belonging to two different phylogenetic clusters, suggesting potential niche adaptation. Using traditional enumeration methods, strains of the two groups were not distinguishable. Reported higher heat resistance of spores of C-*cpe* strains compared with P-*cpe*/non-*cpe* strains was not linked to presence/absence of unique sporulation genes. Moreover, it was found that some P-*cpe* strains can also produce spores with high heat resistance. The impact of these findings for the food industry will be discussed.

O288 Diversity and adaptation of Bacillus cereus spores in the food chain

Frédéric Carlin[1]

[1]INRA PACA Research Center, UMR408 SQPOV Sécurité et Qualité des Produits d'Origine Végétale, Avignon, France

The pathogenic and spore-forming bacteria *Bacillus cereus* accounts for some of the most common foodborne poisonings, occurring under the forms of diarrheal and emetic syndromes due to a range of enterotoxins and to the cyclic peptide cereulide. Reports of *Bacillus cereus* outbreaks in Europe (including very severe emetic outbreaks) markedly increased in recent years. Spores of *B. cereus* are widely dispersed in the environment and contaminate any sort of foods. Assembly of spore structures is the result of a complex process and is strongly influenced by the environment of sporulation, with deep consequences on spore properties. With *Clostridium botulinum* and *C. perfringens*, *B. cereus* is a concern for mildly heat-processed foods because of spore resistance and the ability of many strains to multiply at refrigeration temperature. The phenotypic diversity of *B. cereus* strains has been established long ago. Several descriptions of the genetic structure of the whole group have been provided and now define genetic groups with specific phenotypic characters. With respect to food safety, these groups have different growth temperature profiles, and also differ by their involvement in foodborne poisoning incidents, production of toxins causing poisonings, resistance to heat, adaptation to low pH or to low a_w, etc… The risk for consumers of *B. cereus* foodborne poisonings should be considered with respect to the type of strains present in foods.

O289 Bacillus spore germination and outgrowth; molecular mechanisms and antimicrobial strategies

Wishwas Abhyankar[1], Bhagyashree Swarge[1], Soraya Omardien[2], Juan Wen[3], Raymond Pasman[3], Erik Manders[4], Zhiwei Tu[1], Leo de Koning[5], Chris de Koster[5], Peter Setlow[6], Stanley Brul[7]

[1]University of Amsterdam, MBMFS & MSB @ Swammerdam Institute for Life Sciences, Amsterdam, Netherlands
[2]Akzo Nobel Coatings, Microbiology, Sassenheim, Netherlands
[3]University of Amsterdam, MBMFS @ Swammerdam Institute for Life Sciences, Amsterdam, Netherlands
[4]Confocal, Research, Amsterdam, Netherlands
[5]University of Amsterdam, MSB @ Swammerdam Institute for Life Sciences, Amsterdam, Netherlands
[6]UConn Health, Molecular Biology & Biophysics, Farmington, United States
[3]University of Amsterdam, MBMFS @ Swammerdam Institute for Life Sciences, Amsterdam, Netherlands

Background: Bacterial spores are ubiquitous in nature, can survive food preservation processes and cause, upon outgrowth, food spoilage and human disease. The risk is exacerbated by heterogeneous germination and outgrowth behavior of isogenic spore populations.

Objectives: We discuss approaches aimed at synchronization of *Bacillus subtilis* sporulation, and analyze spore proteins as well as germination and outgrowth physiology. Our studies focus on the characterization of the mechanisms involved in normal spore development and germination.

Methods: We present proteomics results aimed at broadening our knowledge about spore structure dynamics and the identification of putative antimicrobial targets. For relative quantification we used ^{15}N metabolic labelling. Live-imaging and fluorescence microscopy allowed us to analyze spore germination and outgrowth at the single spore level as well as the presence of 'germinosomes' (clustered germination receptors) in *B. subtilis.*

Results: The proteomics analyses showed many energy related proteins necessary for 'kick-starting' life. Most glycolytic enzymes were identified in dormant spores as well as many of the proteins involved in amino acid biosynthesis. Furthermore most germination receptors were identified. Germinosome visualization using receptor protein fusions showed that *B. subtilis* spores often contain two germination protein foci. The germinosome's impact on models of spore germination is being assessed. Finally, we probed the mode of action of human thrombocidin derived peptides TC19 and TC84, possible modifiers of the microbiome. Perturbed membrane fluidity homeostasis was shown to be central to their mode of action. Data acquisition and antimicrobial analyses are being extended to *Bacillus cereus* and the anaerobic pathogen *Peptoclostridium difficile.*

O290 The bacterial protein translocation channel at work

Peter Pohl[1]

[1]*Biophysics, Linz, Austria*

Background: Translocation of many secretory proteins through the bacterial plasma membrane is facilitated by the protein translocation complex SecYEG. The molecular mechanisms of how motor protein SecA, membrane potential or membrane protein folding energize polypeptide translocation is not resolved.

Objectives: We now monitored (i) the assembly of the SecA-SecYEG translocation machinery in a reconstituted system to identify both voltage and ATP dependent steps and (ii) the interactions between translocation intermediates and already translocated membrane helices.

Methods: We used luminescence resonance energy transfer, single molecule fluorescence resonance energy transfer, electrophysiological recordings of single channel events, and high-speed atomic force microscopy to monitor the interactions between the different constituents of the translocon.

Results: The different methods consistently revealed that the initial steps of translocon assembly are ATP-independent. SecYEG's voltage sensitivity is essential to maintain the membrane barrier to ions and other small molecules. In addition to membrane voltage, ion leakage across the activated translocon also depends on the hydrophobicity of the translocation intermediate, i.e. on its preference for the aqueous environment of the channel or the hydrophobic interior of the membrane core. The probability of polypeptide secretion or membrane insertion appears also to be affected by the ability of the stalled polypeptide chain to recognize transmembrane helices outside the translocon.

O291 Integration of membrane proteins: stop and go

Martin Spiess[1]

[1]*University of Basel, Biozentrum, Basel, Switzerland*

Background: Most membrane proteins are inserted into the lipid bilayer by the Sec61/SecYEG translocon as transmembrane α-helices. The translocon provides the nascent polypeptide access to the lipid phase for thermodynamic equlibration. Each polypeptide segment thus appears to autonomously define its integration behavior by its hydrophobicity.

Objectives: The hydrophobicity threshold for membrane integration was tested for the same set of potential transmembrane segments in different contexts to determine the effect of flanking sequences on the integration process.

Methods: Model proteins containing a series of potential transmembrane segments were expressed in yeast (wild-type or mutated in Sec61p) and pulse-labeled with radioactive methionine. Resulting topologies were dentified from the glycosylation patterns.

Results: The sequence context affects the hydrophobicity threshold of transmembrane segment integration in unexpected ways. In particular, the folding properties of the sequence upstream of the potential transmembrane sequence influence the partitioning process, which is part of a dynamic process. The results shed a new light on membrane protein design.

O292 Protein transport in bacteria: multiple pathways and common concepts

Hans-Georg Koch[1]

[1]*Institute for Biochemistry and Molecular Biology, Medicine, Freiburg, Germany*

Background: Compartmentalization is a unifying principle of eukaryotic and prokaryotic cells and necessitates specific protein delivery systems that transport proteins from their site of synthesis in the cytosol to their sites of function. Bacteria use a large variety of protein delivery systems in parallel to transport proteins to the cell envelope and into the extracellular environment.

Objectives: The SecYEG translocon acts at the center of the bacterial protein transport and is engaged by two distinct targeting pathways: the SRP-dependent pathway, dedicated mainly to inner membrane proteins, and the SecA-dependent targeting of secretory proteins. Conceptually, both pathways are considered to operate by distinct mechanisms: SRP targets its substrates co-translationally, while SecA targets its substrates after their synthesis is completed, i.e. post-translationally.

Methods: in vitro transcription/translation systems; in vivo and in vitro site-directed cross-linking, mass spectrometry, mRNA targeting, single-molecule imaging

Results: Our data show that bacterial protein delivery systems are by far more versatile than initially anticipated. SRP can operate in a post-translational targeting mode and SecA can interact with its substrates co-translationally. In addition, bacteria also use translation-independent targeting processes for the membrane enrichment of mRNAs encoding for membrane proteins. Our data furthermore highlight the importance of a highly dynamic SecYEG translocon for transporting substrates delivered by different targeting pathways.

O293 Microbial formate-nitrite (lactate) transporters: mechanism and potential as novel drug target

Eric Beitz[1]

[1]*Pharmaceutical Institute, Pharmaceutical and Medicinal Chemistry, Kiel, Germany*

Background: Due to still failing vaccination and rapidly growing resistance, new antiprotozoal drugs and novel modes of action are urgently needed. We have discovered a novel approach to attack the parasites at their periphery and kill them by blocking transporters of the major end product of the vital energy metabolism, i.e. lactic acid. These transporters belong the exclusively microbial formate-nitrite transporter, FNT, family.

Objectives: We aim at determining structure-function relationships of the FNT-family, elucidate substrate selectivity filters and the transport mechanism; eventually, we want to find more sustainable drugs to fight resistant parasites causing – among others – toxoplasmosis, amoebiasis, and malaria.

Methods: We express FNTs in yeast or a cell-free transcription/translation system for transport assays. We introduce mutations and analyze transport under various conditions. We phenotypically and biophysically screen for drug-like inhibitors and synthesize expansion libraries.

Results: We characterized FNTs from *Plasmodium falciparum, Entamoeba histolytica*, and *Toxoplasma gondii*. We discovered FNT inhibitors that kill malaria parasites and *T. gondii*. Currently, we are expanding our work to other pathogens. We further identified a general FNT substrate selectivity filter, which is reminiscent of the aquaporin aromatic/arginine region regarding its localization within the FNT protein, its amino acid composition, and size-exclusion function. Finally, we elucidated the basic FNT transport mechanism: an invariant lysine attracts the substrate anion into the transporter vestibule, the entering substrate encounters an increasingly hydrophobic environment initiating dielectric pK_a disturbance and proton transfer from the water bulk for transport of the neutral substrate species. We term this mechanism the “dielectric slide”.

O294 The giant viruses: history, recent developments, enigmas and promises of a new branch of Virology

Jean-Michel Claverie[1]

[1]*Aix-Marseille University & CNRS, Laboratoire Information Génomique et Structurale (IGS) UMR7256, Marseille, France*

Background: The saga of "giant viruses" (viruses with cell-like particle and genome sizes) started in 2003 with the publication of Mimivirus, the prototype of the Mimiviridae family. With its double-stranded DNA genome of more than one megabase encoding about 1000 proteins, the Acanthamoeba-infecting Mimivirus initiated a revolution in virology, showing that an entire part of the virosphere was ignored due to flawed historical protocols and concepts.

Objectives: For 15 years, several laboratories performed a planetary wide sampling of fresh, brackish, and marine waters, as well as various soils, from temperate to arctic and antarctic regions.

Methods: Our protocol initially involves the co-cultivation of samples with Acanthamoeba in search of lytic viruses. Following the cloning and population expansion of giant virus isolates, they were fully characterized using genomics, transcriptomics, proteomics, and their infectious cycle documented using electron microscopy. More recently, several groups attempted to speed up the discovery process using metagenomic analyses of aquatic environments and soils.

Results: Fifteen years of endeavour have led to 3 distinct families of giant viruses, in addition to the Mimiviridae. These proposed families are the Pandoraviridae, the Pithoviridae, and the Molliviridae. The prototypes of the two later families have been revived from 30.000 year-old siberian permafrost, demonstrating the capacity of large DNA viruses to remain infectious since the Neanderthal era. A puzzling feature shared by all giant viruses is the large proportion of orphan proteins they encode, raising fundamental questions about their evolutionary origin and their relationship with the cellular Tree of Life

O295 Ultrastructure of Meelsvirus, a New Prototype of "Giant Virus" Infecting Epidermal Cells of Arrow Worms (Phylum Chaetognatha)

George Shinn[1], Brianna L. Bullard[2]

[1]*Truman State University, Department of Biology, Kirksville, Missouri, United States*
[2]*University of Nebraska, School of Biological Sciences, Lincoln, Nebraska, United States*

Background: Most known giant viruses, i.e., viruses producing virions exceeding 0.3 microns, parasitize amoebae. These viruses vary considerably in their dependence on host nuclear functions and in the structure and molecular composition of virions, but their virions all self-assemble within the host's cytoplasm. *Meelsvirus* infects epidermal cells of the planktonic predatory arrow worm, *Adhesisagitta hispida*. It has unique bowling pin-shaped virions that self-assemble within host cell nuclei.

Objectives: To establish *Meelsvirus* as new prototype of giant virus, we analyzed the ultrastructure of mature virions and structural apsects of self-assembly.

Methods: Sections of infected cells were examined using conventional transmission electron microscopy.

Results: Infected cells occur in patches and have greatly hypertrophied nuclei. Mature virions are suspended throughout the nuclei in an abundant electron-lucent nucleoplasm. The ovoid nucleocapsids average 0.53 micron long and the 31 nanometer-thick capsid wall is composed of very large, radially arranged, complexly shaped capsomers. A thin surrounding envelope extends away from the nucleocapsid to form a large conical "tail" filled with electron-dense material. Entire virions average 1.25 microns long. New virions arise from the surface of peripheral electron-dense nucleoplasm. The capsid and envelope assemble coincidently and capsids begin filling well before the wall is complete. We hypothesize that infection of new hosts occurs by rupture of protruding virion-filled nuclei when the infected arrow worms mate. Presence of empty naked capsids in the cytoplasm of infected cells suggests that the envelope and tail participate during infection. Genomic work is needed to determine the phylogenetic position of *Meelsvirus*.

O296 Genome analysis of the marine flagellate Cafeteria roenbergensis suggests high mobility of virophages

Matthias Fischer[1], Thomas Hackl[2]

[1]*Max Planck Institute for Medical Research, Biomolecular Mechanisms, Heidelberg, Germany*
[2]*Massachusetts Institute of Technology, Department of Biology, Cambridge, MA, United States*

Background: Virophages are DNA viruses of protists that depend on a coinfecting giant virus for their replication, and can increase host survival in the presence of a lytic giant virus. The mavirus virophage is able to integrate into the genome of its host, the marine protozoan *Cafeteria roenbergensis*. A host protective role has been suggested for integrated mavirus virophages, but conclusive data are missing.

Objectives: Prompted by the efficient host genome integration of mavirus, we addressed the prevalence and diversity of endogenous virophages in natural populations of *C. roenbergensis* to gain insight into host-virophage coevolution.

Methods: We sequenced and assembled the genomes of four *C. roenbergensis* strains using PacBio and Illumina platforms and screened them for endogenous virophages.

Results: Each of the four flagellate genomes contained dozens of endogenous mavirus-like elements (EMALEs), which comprise 1-2% of these genomes. We characterized in detail 32 complete EMALEs that were flanked by terminally inverted repeats and characterized by significantly lower GC contents (29-51%) than the host genome (70%). Some EMALEs were presumably intact, others displayed fragmented genes. Some EMALEs were shared by several host strains, others were exclusive to a given host genome. We found a slight integration site preference for repetitive genome regions, although no conserved DNA sequence motif surrounding the EMALEs was identified. Nine of the 32 EMALEs were interrupted by tyrosine recombinase-encoding retrotransposons of the NGARO group. Our results suggest a long-standing and highly dynamic interaction between mavirus-like virophages and their protozoan hosts, with profound consequences for host genome architecture.

O297 Viroids: how a minimal non-protein-coding RNA initiates disease in its natural host

Ricardo Flores[1], Beatriz Navarro[2]

[1]*Instituto de Biologia Molecular y Celular de Plantas (UPV-CSIC), Molecular and Evolutionary Virology, València, Spain*
[2]*Institute for Sustainable Plant Protection (CNR), University of Bari, Italy*

Background: Viroids are non-protein-coding RNAs of 246-434 nt infectious to certain plants wherein they replicate in the nucleus (family *Pospiviroidae)* or in plastids (family *Avsunviroidae)*. Viroids appear very suitable systems to address the question of how viral and sub-viral agents initiate disease.

Objectives: Plants react to viroids activating RNA interference (RNAi). Such defensive response has been involved in pathogenesis, but whether it is the initial trigger, or just mediates intermediate-late steps, remains unsolved. We have tackled this issue using peach latent mosaic viroid (PLMVd, *Avsunviroidae),* some variants of which cause an extreme chlorosis (peach calico, PC) or a yellow mosaic (PYM).

Methods: Starting from symptomatic and asymptomatic leaf sectors we have: i) cloned, sequenced and bioassayed full-length PLMVd-cDNAs and analyzed their progeny, ii) deep-sequenced the RNAi-derived PLMVd small RNAs (sRNAs), predicted bioinformatically their host mRNA targets, and verified their cleavage at the expected positions by RNA ligase-mediated RACE and their lower accumulation in symptomatic sectors by RT-qPCR.

Results: In contrast with PC, associated with a 12-13-nt insertion, PYM determinant maps at one single nucleotide. Moreover, 21-nt PLMVd-sRNAs containing the PC-associated insertion and the sole PYM change guide, respectively, cleavage of the mRNAs encoding the chloroplastic heat-shock protein cHSP90 and a thylakoid translocase subunit, both required in chloroplast biogenesis. PLMVd-sRNAs triggering PC and PYM have 5'-terminal Us, thus involving Argonaute 1 (with a major defensive role in RNAi) in what likely are the primary alterations eliciting different chloroses, which thus appear early and specific symptoms rather than late and non-specific effects.

O298 Ensuring the enduring relevance of the International Code of Nomenclature of Prokaryotes

Iain Sutcliffe[1]

[1]*Northumbria Univeristy, Applied Sciences, Newcastle, United Kingdom*

Background: Microbial systematics is at a cross-roads, with methods that have served well for half a century challenged by disruptive technologies. The application of culture-independent methods for characterisation of microbial communities has led to spectacular advances in our understanding of the diversity and scale of microbial life. In contrast to the high volumes of data being generated through such high throughput approached, the traditional 'polyphasic' taxonomic approach remains labour intensive and low throughput.

Objectives: It seems inevitable that fundamental changes are needed to the framework within which microbial taxonomists work.

Methods: There is a clear the need for the International Committee on Systematics of Prokaryotes to provide effective leadership regarding the process for naming microbial taxa, as defined in the International Code of Nomenclature of Prokaryotes (ICNP). It is necessary that research communities beyond the relatively narrow field of microbial taxonomy are aware of the importance of sustaining the historic success of the ICNP, which acts as a barrier to nomenclatural anarchy. To ensure this success continues, it is vital that the ICNP is maintained as an accessible and functional 'living document' that is fit for purpose.

Results: Addressing these challenge are also an opportunity: if handled well the fundamental significance of taxonomy as a bedrock for disciplines such as microbial ecology can be restored. This is important if we are to ensure that capable and energetic early career scientists are attracted into the field.

O299 Database-driven bacterial classification system for clinical and microbiome-related microbiology

Jongsik Chun[1]

[1]*Seoul National University & ChunLab, Inc., School of Biological Sciences, Seoul, Korea, Rep. of South*

Background: Prokaryotic taxonomy has benefited from the successful introduction of high-throughput and cost-effective DNA sequencing technologies. Given the generally accepted consensus on the species concept of prokaryotes, we have great opportunity to improve and facilitate the current system of classification and nomenclature, which serves a crucial backbone for most microbiological disciplines. The International Code of Nomenclature of Prokaryotes (ICNP) plays an essential role in maintaining the integrity and stability with minimum requirements for validation of taxonomic names. Even so, there are significant delays between the formal description of new taxa and their usage in clinical and microbiome-related microbiology.

Objectives: To develop an integrated taxonomic database that reflects up-to-date prokaryotic diversity in public genome databases

Methods: We used the genome-based identification system (www.truebacid.com) to recognize new species hidden in public databases and assigned them as genomospecies with tentative names.

Results: One of the advantages of using genome-based identification scheme is to automatically recognize new species with confidence given that there are type strain genome data with sufficient taxonomic coverage. We have developed a database system (http://www.ezbiocloud.net) of genome-based classification with tentative nomenclature that allows timely recognition of existing diversity in various environments. This interactive and trackable system allows the large-scale profiling and monitoring of microbiome and infectious diseases.

O300 Genome -based characterization of taxa in Burkholderia sensu lato

Marike Palmer[1], Emma Steenkamp[1], Paulina Estrada de los Santos[2], Juan Carlos Villalobos-Rocha[3], Euan James[4], Stephanus Venter[1]

[1]*University of Pretoria, Biochemistry, Genetics and Microbiology, Pretoria, South Africa*
[2]*Instituto Politécnico Nacional, Escuela Nacional de Ciencias Biológicas, Ciudad de México, Mexico*
[3]*Instituto Politécnico Nacional , Departamento de Microbiología, Ciudad de México, Mexico*
[4]*The James Hutton Institute, Invergowrie, United Kingdom*

Background: The bacterial group *Burkholderia sensu lato*, is a large and complex assemblage that consists of members with a wide variety of lifestyles, ranging from pathogens through to beneficial bacteria with immense biotechnological potential. Due to the diverse nature of its members, the taxonomic standing of this group has recently been interrogated in multiple studies in order to provide a more comprehensive and natural circumscription of the genera contained within this group.

Objectives: As the description of these genera were mainly based on phylogenetic cohesion inferred from limited data, a robust phylogeny from all shared genomic information was constructed for this assemblage. We investigated the inferred phenotypic and biological traits, unique or shared among the genera, which could support the current taxonomic classification of these lineages.

Methods: Shared gene sets for the larger *Burkholderia sensu lato* group was identified with the EDGAR server and a super matrix approach was followed to construct a robust Maximum Likelihood phylogeny. Genome comparisons were then performed to identify lineage specific genes and functionally annotated with the aid of the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. This allowed for the reconstruction of metabolic pathways that were shared among some, or unique to, the lineages representing genera within this group.

Results: Various characteristics associated with specific lifestyles was identified, e.g. the metabolism of fungal compounds found in members of *Mycetohabitans*. It was also observed that some reactions were performed by non-orthologous proteins between the different lineages, suggesting homoplasious acquisition of these traits.

O301 Bacterial taxonomy in the age of high throughput sequencing

Anne Willems[1]

[1]*Ghent University, Dept. Biochemistry and Microbiology, Ghent, Belgium*

Background: Over the years, bacterial systematics has evolved hand-in-hand with technological developments from purely morphology and biochemistry-based taxonomy over polyphasic taxonomy towards genome-based taxonomy. Where bacterial taxonomy traditionally was based on pure cultures, with an important place for type strains, developments in sequencing technology now allow direct assessment of genome diversity without cultivation. The wide application of 16S rRNA gene sequencing in ecological studies of all kinds of environments in recent years has exposed the limits of our current knowledge and revealed the extent of the challenge to document all prokaryotic diversity which is dominated by the so-called microbial dark matter of uncultivated taxa.

Objectives: It is clear that current taxonomic practice to describe cultivated bacteria, already including a genome sequence of type strains, but still requiring chemotaxonomic differentiation, is not practical, nor appropriate to classify the enormous amount of uncultivated diversity that sequencing studies are revealing. As a consequence several calls and proposals to move towards a genome-based prokaryote taxonomy, for cultivated as well as uncultivated diversity, have been published.

Methods: In this contribution the taxonomic challenges of microbial community-based ecological surveys and cultivation-based prokaryote studies will be discussed using examples from our research on various environmental microbiomes.

Results: The combination of these approaches represents new opportunities for powerful synergies towards cultivating previously uncultured prokaryotes and experimentally documenting their metabolic capacities and ecological significance.

O302 First Contact: exploring the role of the early life microbiota

Lindsay Hall[1]

[1]*Quadram Institute Bioscience, Norwich Research Park, Norwich, United Kingdom*

The human microbiome has emerged as a central player in human health and wellbeing. Importantly, 'first contact' between microbes and their host represents a critical developmental window in which the foundations for lifelong health are laid down. Indeed, it is now recognised that disturbing this fledgling microbial ecosystem has both short- and long-term consequences such as increased risk of infection and chronic inflammatory diseases. Thus, understanding the factors that modulate the microbiome during the first stages of life, during pregnancy, and in infancy, is a key research focus. In this WH Pierce Prize lecture I will discuss my career journey and how this has led to the labs current research focus involving defining interactions of the gut microbiota with the host during the early life developmental window. Specifically, I will cover our clinical work relating to beneficially modulating the preterm infant microbiome, and our mechanistic studies using model systems to determine mode-of-action for specific microbiota members (i.e. *Bifidobacterium*), with a key focus on developing novel live biotherapeutics for improving human health.

PM001 L-Rhamnose metabolism in Clostridium beijerinckii strain DSM 6423

Mamou Diallo[1], Andre Simons[1], Servé Kengen[2], Ana López-Contreras[1]

[1]Food & Biobased Research, Biobased Research, Wageningen, Netherlands
[2]Wageningen University & Research, Department of Agrotechnology and Food Sciences, Wageningen, Netherlands

Background: Macroalgae (or seaweeds) are considered potential biomass feedstocks for the production of renewable fuels and chemicals. Their sugar composition is different from that of lignocellulosic biomasses, and in green species, including *Ulva lactuca*, the major sugars are L-rhamnose and D-glucose. *C. beijerinckii* DSM 6423 utilized these sugars in a *U. lactuca* hydrolysate to produce acetic acid, butyric acid, isopropanol, butanol, and ethanol (IBE), and 1,2-propanediol. D-Glucose was almost completely consumed in diluted hydrolysates, while L-rhamnose or D-xylose was only partially utilized.

Objectives: In this study, the metabolism of L-rhamnose by *C. beijerinckii* DSM 6423 was investigated to improve its utilization from natural resources.

Methods: Fermentations on D-glucose, L-rhamnose, and a mixture of D-glucose and L-rhamnose were performed. Genome and transcriptomics analysis of D-glucose- and L-rhamnose-grown cells were used to further study the L-rhamnose metabolism in *C. beijerinckii* DSM 6423.

Results: On L-rhamnose, the cultures showed low growth and sugar consumption and produced 1,2-propanediol, propionic acid, and *n*-propanol in addition to acetic and butyric acids, whereas on D-glucose, IBE was the major product. On a D-glucose–L-rhamnose mixture, both sugars were converted simultaneously and L-rhamnose consumption was higher, leading to high levels of 1,2-propanediol (78.4 mM), in addition to 59.4 mM butanol and 31.9 mM isopropanol. Genome and transcriptomics analysis of D-glucose- and L-rhamnose-grown cells revealed the presence and transcription of genes involved in L-rhamnose utilization but also in bacterial microcompartment (BMC) formation.

PM003 A marine bacterium, bacillus sp. isolated from the sediment samples of algoa bay in south africa produces a polysaccharide-bioflocculant

Ncedo Ntozonke[1], Uchecwukwo Nwodo[1], anthony okoh[1]

[1]*University of Fort Hare, Biochemistry and Microbiology, Alice, South Africa*

Background: Bioflocculants mediate the removal of suspended particles from solution. Apart from the merits of biodegradability and harmlessness, bioflocculants could be viable as industrially relevant flocculants as they are a renewable resource. Additionally, the shortcomings associated with the conventionally used flocculants such as aluminium salts and acrylamide polymers, which include dementia and cancer, highlight more the need to use bioflocculants as an alternative.

Objectives: The imperative of identifying bioflocculants has propelled the exploration of extreme environments seeking microbial species with enhanced bioflocculant production potential and as well with high flocculation efficiencies

Methods: In this study a marine sediment bacterial isolate was screened for bioflocculant production. The identity of the strain was authenticated through Basic local alignment search tools (BLAST) analysis of 16S ribosomal deoxyribonucleic acid (rDNA). Culture conditions and nutritional requirements were optimized, and production of the bioflocculant by the bacterium was characterized

Results: (BLAST) analysis of 16S rDNA) sequence of the bacterial isolate showed 98% similarity to Bacillus thuringiensis MR-R1. The bacteria produced bioflocculant optimally with inoculum size (4% v/v) (85%), glucose (85.65%) and mixed nitrogen source (urea, ammonium chloride and yeast extract) (75.9%) and the divalent cation (Ca2+) (62.3%). Under optimal conditions, a maximum flocculating activity of over 85% was attained after 60 h of cultivation. The purified polysaccharide-bioflocculant flocculated optimally at alkaline pH 12 (81%), in the presence of Mn2+ (73%) and Ca2+ (72.8%). The high flocculation activity shown indicates that the bioflocculant may contend favourably as an alternative to the conventionally used flocculants in water treatment.

PM004 studies on anti-cancerous enzyme arginine deiminase isolated from pseudomonas furukawaii

Rakhi Dhankhar[1], Sanjay Kumar[1], Pooja Gulati[1]

[1]*Maharshi Dayanand University, Rohtak, Haryana, India, Microbiology, Rohtak, India*

Background: The rapid growth of tumors results in decreased expression of certain enzymes leading to auxotrophy for some amino acids. This difference in metabolism is harnessed in amino acid deprivation therapy (AADT) and the auxotrophic tumors are specifically targeted by amino acid depleting enzymes like arginine deiminase (ADI).

Objectives: A robust ADI producing bacterium with higher activity at physiological conditions was isolated and identified. Further production of native enzyme was optimized and ADI gene was cloned and over-expressed in *Escherichia coli* for anticancer studies.

Methods: Enrichment method was used to isolate ADI producing bacteria from pond water and soil. Qualitative and quantitative screening was performed. The best producer was identified by 16S rRNA. ADI production was optimized using one-factor- at –a- time approach. The ADI gene (arcA) was over-expressed in *E. coli.* rADI was purified and tested for anti-cancerous activity.

Results: Of the 143 isolates, *Pseudomonas furukawaii* was identified as the best ADI producer. A three -fold increase in activity was observed after optimization. arcA gene (1251 bp) was cloned and expressed in pET28 a(+) in *E. coli* BL21. His-tagged rADI (~ 46kda) was confirmed by western blotting. rADI was purified using Ni^{2+}-NTA chromatography and exhibited anti-cancer activity against HepG2 cell lines.

This study reported a novel source of ADI which can serve as a potential candidate for development of more efficient and stable anti-cancerous drug.

PM005 Production of arsenic-binding siderophore heterobactin B in arsenic-stress conditions

Gerardo Retamal-Morales[1], Ringo Schwabe[2], Marika Mehnert[2], Dirk Tischler[3], Michael Schlomann[2], Gloria Levicán[1]

[1]*Universidad de Santiago de Chile, Biology, Santiago, Chile*
[2]*Freiberg University of Mining and Technology, Biosciences, Freiberg, Germany*
[3]*Ruhr-Universität Bochum, Microbiology, Bochum, Germany*

Background: Inorganic arsenic species are highly toxic pollutants produced by anthropogenic sources, which generates an environmental problem in several countries including Chile and Germany. Thus, a constant improvement in the current decontamination technologies is needed. *Rhodococcus erythropolis* S43 is an arsenic-tolerant actinobacterium isolated from an arsenic contaminated soil which has shown the capacity to produce arsenic-binding metabolites when cultured in iron-depleted medium.

Objectives: Characterize the arsenic-binding siderophores produced by *R. erythropolis* S43 and determine their participation against arsenic-stress.

Methods: To induce siderophore production the strain was cultured in iron-free M9 liquid media in presence of sodium arsenite 937 µM, and tested using the colorimetric method CAS assay to evaluate iron-chelating capacity of siderophores, the arsenic-binding capacity was determined using As-$_m$CAS assay, a modified version of the traditional CAS. The chemical characterization of the binding compounds was carried out by HPLC, ^{1}H-NMR and LC-MS, while the expression of the siderophore biosynthesis gene, *htbG*, was evaluated by RT-qPCR

Results: The strain S43 has a putative siderophore production cluster *htbABCDEFGHIJK*, previously characterized for heterobactin production. The metabolites produced by S43 showed iron and arsenic-binding capacity, achieving an activity equivalent to 10 mM of desferroxamine-B, while the chemical characterization of the binding compounds showed the presence of the mixed-type siderophore heterobactin B, which was responsible of the iron and arsenic-binding activity. Additionally, a study of the transcript level of the gene *htbG* showed that arsenic-stress does not affect its expression, therefore, the production of the arsenic-binding heterobactin B could be independent of arsenic-tolerance.

PM006 Development and evaluation of bio-based stabilisers for potential use in unpaved road construction and maintenance

Veshara Malapermal[1;2], Rajesh Lalloo[1], Prisha Naicker[1], Martin Mgangira [3], Samson Mukaratirwa[2], Santosh Ramchuran [1;2]

[1]*CSIR, Biosciences , Pretoria, South Africa*
[2]*University of Kwazulu-Natal, Microbiology , Durban, South Africa*
[3]*CSIR Built Environment, Pretoria, South Africa*

Background: The construction industry is exploring innovative opportunities involving biological products to modify the structural properties of subsurface soil, in terms of strength, volume stability, durability and permeability through novel engineering techniques. This is enabled by interdisciplinary research at the confluence of biotechnology, civil engineering and geochemistry. An integrated approach has the potential to meet society's ever-expanding needs for innovative methods to improve soil, develop new construction materials and enhance support for existing infrastructure. Several microorganisms and other existing bio-based products such as secondary metabolites, enzymes, endospores and extracellular polymeric substances have been considered as potential alternatives to conventional chemical stabilisers for development of sustainable road infrastructure. However, the primary challenge with this bio-based approach has been the isolation and selection of appropriate microorganisms due to the lack of established medium to high throughput screening to perform strength testing methodologies at bench scale.

Objectives: This study focused on evaluating an isolated *Bacillus* strain for use as a bio-stabiliser using in-house developed *in vitro* miniaturised strength tests.

Methods: The miniaturised test equipment was successfully developed using computer-aided design and 3D printing technologies which allowed various performance evaluations and validation trials to be conducted on selected soil types and fly ash.

Results: Using these systems, biological components were rapidly evaluated to enable selection of component types of a base case target *Bacillus* species. This study forms the basis for *in vitro* selection methodology and the performance assessment to enable the development of improved biological stabiliser products for application in the construction industry.

PM007 Effects of DNA extractoin method on microbial profiling of drinking water by NGS of 16S rRNA

Aleksandra Knezev[1], Dennis Kloes[1], Maja Taucer-Kapteijn[1], Eric Penders[1], Ruud Steen[1]

[1]*Het Waterlaboratorium N.V., Haarlem, Netherlands*

Background: Various commercially available kits for extraction of nucleic acids (NA), based on (bio)chemical or mechanical cell-disruption are applied during microbial profiling of drinking water by NGS of 16S rRNA. NA extraction might affects the results and further standardisation is required for microbial profiling of drinking water.

Objectives: Select optimal NA-extraction method for for NGS of 16S rRNA.

Methods: Samples**:** 2 l of drinking water, filtered over 0,22 µm polycarbonate filter, stored 24-h at 4°C. Internal control (IC, ZymoBIOMICS™ Microbial Community) and blank (NA-free water). DNA extraction: chemical cell-disruption: Nuclisense (Biomerieux), Chemagic Bacteria DNA (Perkin Elmer) Magcore (GCbiotech); mechanical cell-disruption: MOBIO DNeasy Powerbiofolim KIT (Qiagen). Each NA-extraction kit is tested in triplicate. The extracted NA is kept at -20°C. Microbial profiling: v4 region of 16S rRNA (515F-806R or 515F-909R) sequenced on Illumina MiSeq sequencer (v2 Kit). Data were analysed with MOTHUR (Schloss, Westcott et al. 2009).

Results: A total of 988 OTU's were detected in drinking water. 605 OTU's were common for all extraction methods. OTU's with abundance above 0,3% represented 79-82% of the community and were common regardless the cell-disruption type. Mechanical cell-disruption resulted in highest number of OTU's (649, rel. error 2%) with 44 exclusive OTU's at abundance 0,03-0,25%. Relative abundance of gram-positive genera in IC (Lysteria and Enterococcus) was lower for chemical than for mechanical cell-disruption. DNA extraction method affects the results of microbial profiling at lower abundance level for the OTU's <0,3%. Mechanical cell-disruption provides better NA yields for all microorganisms when compared to chemical cell-disruption.

PM008 The membranous mechanisms of antibacterial effects of iron oxide nanoparticles

Lilit Gabrielyan[1], Armen Trchounian[1]

[1]*Russian-Armenian Slavonic University, Medical Biochemistry and Biotechnology, Yerevan, Armenia*

Background: Nanoparticles (NPs) can be used as antibacterial agents, as they show antimicrobial activity against different pathogens. However, growth inhibition and mechanisms of these effects have to be studied.

Objectives: The effects of iron-oxide NPs on the growth and membrane properties such as membrane proton (H^+) conductance, H^+-flux through membrane, and the F_OF_1-ATPase activity in Gram-negative *Escherichia coli* two strains K-12 and BW25113 have been investigated.

Methods: Iron-oxide NPs (average size of 10.64±4.73 nm) in a concentration 50–250 μg/mL were added into the growth medium. H^+ conductance was evaluated by so-called "acid pulse" technique; the H^+-flux was measured using selective electrode; the ATPase activity was determined by amount of inorganic phosphate liberated after adding ATP to membrane vesicles.

Results: NPs showed inhibitory effects on the bacterial growth; *E. coli* K-12 showed more sensitivity than BW25113. NP's antimicrobial activity was also revealed in a decrease of the colony forming units. NPs at concentration of 100 μg/mL increased the H^+ conductance by ~1.5-fold, at the same time decreased the energy-dependent H^+-fluxes in *E. coli* both strains (~2.0-fold in K-12). H^+-fluxes were also decreased in the presence of N,N'-dicyclohexylcarbodiimide (DCCD), inhibitor of the F_OF_1-ATPase, which might be a target for NPs. ATPase activity has been increased by addition of NPs even in the presence of DCCD. The antibacterial activity of iron-oxide NPs is a result of their interaction with bacterial membranes, causing membrane damage and inactivation of bacteria. In this case iron-oxide NPs can be promising antibacterial agents for biomedical applications.

PM009 Antibacterial activity of Sanky (Corryocactus brevistylus) methanol extract against Staphylococcus aureus and Enterococcus faecalis.

Ronald Aquino Ortega[1;2], Hugo Carrillo-Ng[3;4], Luz Paucar Menacho[5], Miguel Angel Aguilar-Luis[2;6], Juana del Valle-Mendoza[6]

[1]*Universidad Peruana de Ciencias Aplicadas, Peru*
[2]*Instituto de Investigación Nutricional, Lima, Peru*
[3]*Instituto de Investigación Nutricional, Peru*
[4]*Universidad Peruana Cayetano Heredia, Lima, Peru*
[5]*Universidad Nacional del Santa, Chimbote, Peru*
[6]*Universidad Peruana de Ciencias Aplicadas, Lima, Peru*

Background: Antibiotic resistance and nosocomial infections are a big concern in public health worldwide. *Staphylococcus aureus* and *Enterococcus faecalis* are two important pathogens associated with nosocomial infections. In 2017, the WHO published a list of bacteria for which new antibiotics are urgently needed, which included both bacteria in the highest priority group among gram positive bacteria. *Corryocactus brevistylus*, is a peruvian cactacea with antioxidant properties that grows in the Andes, however, its antibacterial effect has not been studied yet.

Objectives: To determine the antibacterial effect of the methanol extract of the *Corryocactus brevistylus* fruit (Sanky) against *Staphylococcus aureus* (ATCC 12598) and *Enterococcus faecalis* (ATCC 29212).

Methods: The fruits of *Corryocactus brevistylus* were pulverized, soaked with methanol (1:2, w/v) and stored for 7 days. The antibacterial effect against *Staphylococcus aureus* and *Enterococcus faecalis* were evaluated using the cup-plate agar diffusion method by preparing wells with the experimental solutions cultivated in aerobic conditions for 24 h at 37 °C . Six independent tests were performed for each type of bacteria, using penicillin as positive control. The MIC was determined using the microdilution method as described by the CLSI.

Results: Antibacterial effect of the methanol extract was observed with inhibition halos of 23.33 ± 0.72 mm and 24.34 ± 0.55 mm against *Staphylococcus aureus* and *Enterococcus faecalis*, respectively. Meanwhile, penicillin (10 U) showed an inhibition halo of 30.92 mm. The minimum inhibitory concentration of the fruit extract was 831.25 mg/mL for *Staphylococcus aureus* and 207.81 mg/mL for *Enterococcus faecalis.*

PM010 An enzymatic process to produce wide diversity inulin-type microbial fructooligosaccharides

Luz Cristina Vallejo García[1], María Elena Rodríguez Alegría[1], Agustín López-Munguía[1]

[1]*Instituto de Biotecnología, UNAM, Ingeniería Celular y Biocatálisis, Cuernavaca, Mexico*

Background: Inulin and fructooligosaccharides (FOS) have health effects and physicochemical properties that make them food additives with growing demand. These compounds can be found in 15% of plants with flower but also are produced by a wide diversity of bacteria; nevertheless, the market is covered almost completely by extracts from plants. Our group has isolated and characterized several microorganisms and enzymes capable to synthesize inulin and/or FOS from sucrose, among them, the *Leuconostoc citreum* CW28 strain from *pozol*, a non-alcoholic fermented corn beverage of mayan origin

Objectives: We explore the application of inulosucrase (IslA) from *Leuconostoc citreum* CW28, capable to produce high molecular weight inulin (average MW 2600 KDa) together with a commercial endoinulinase (Novozym 960) from *Aspergillus niger* for sequential or simultaneous reaction to synthesize fructooligosaccharides from sucrose

Methods: Whole *Leuconostoc citreum* CW28 freeze dried cells were used as enzymes with sucrose as substrate in sequential and simultaneous reactions with endoinulinase at several ratios and the products profile were analyzed looking for the higher substrate conversion and wider FOS variety

Results: The simultaneous reaction resulted in a higher substrate conversion and a wide variety of FOS as compared to a sequential reaction or the hydrolysis of chicory inulin with endoinulinases. A proteomic analysis of the endoinulinase preparation revealed an additional enzymatic activity, as besides endoinulinase, a fructosyltransferase responsible for the synthesis of FOS from sucrose is present. Consequently, the range of products obtained in reactions combining inulosucrase, fructosyltransferase and endoinulinase with sucrose as substrate may be extended and regulated

PM011 Investigating the potential of the plant growth promoting isolate Pantoea agglomerans DUS1-2 to reduce stress towards Brassica napus L. (canola) in elevated metal environments

Aydin Enez[1;2], Lee Hudek[1;2], Damien Callahan[2], Lambert Brau[1;2]

[1]*Centre for Regional and Rural Futures, Geelong, Australia*
[2]*School of Life and Environmental sciences, Geelong, Australia*

Background: Irrigation of cropped plants changes the distribution and bioavailability of metals in soil. Fluctuations in metals levels, particularly elevated levels, negatively affect plant development from germination through to plant maturation, flowering and seed production (reduced seed quality and quantity). The presence of microbes in the soils of irrigated cropped plants may help buffer the fluctuations in metal levels and offer a safeguard for plants from elevated levels.

Objectives: The research presented here, investigates if the soil isolate *Pantoea agglomerans* DUS1-2 can reduce metal toxicity for *Brassica napus* L. (canola) exposed to deleterious levels of As, Cd, Co, Cu, Fe, Mn, Ni and Zn.

Methods: Inductively coupled plasma mass spectrometry (ICP-MS), quantitative real-time PCR (qRT-PCR), liquid chromatography mass spectrometry (LC-MS), gas chromatography mass spectrometry (GC-MS), and flow cytometry were used to determine the potential for soil microbes to protect cropped plants from elevated soil metal levels.

Results: The metal uptake and stress response mechanisms at the cellular and molecular levels of both plants and bacteria individually and when co-cultured under the different metal treatments were assessed and the effects of inoculation on reducing the plant stress determined. Ultimately, understanding how soil microbes can influence metal bioavailability and reduce stress towards cropped plants is key to the development of targeted microbial inoculants that may increase plant yields and reduce cultivation costs under these conditions.

PM012 Using fish-gut Bacillus to prevent aquaculture fish diseases

Rafaela A. Santos[1;2;3;4], Aires Oliva-Teles[1;2], Maria José Saavedra[2;3;4;5], Paula Enes[2], Cláudia R. Serra[2;3;4]

[1]*Faculdade de Ciências, Universidade do Porto, Biologia, Porto, Portugal*
[2]*CIIMAR - Centro Interdisciplinar de Investigação Marinha e Ambiental, Matosinhos, Portugal*
[3]*CITAB - Centro de Investigação e Tecnologias Agroambientais e Biológicas, Universidade de Trás-os-Montes e Alto Douro, Vila Real, Portugal*
[4]*CECAV – Centro de Ciência Animal e Veterinária, Universidade de Trás-os-Montes e Alto Douro, Vila Real, Portugal*
[5]*Universidade de Trás-os-Montes e Alto Douro, Departamento de Ciências Veterinárias, ECAV, Vila Real, Portugal*

Background: Bacterial diseases outbreaks are a major constraint in aquaculture, an industry responsible for more than 50% of global seafood consumption. Their emergence is also associated with a misuse of antibiotics, posing serious threats to public health. One promising disease-preventive strategy is the use of probiotics. *Bacillus* species are the most attractive probiotics for aquaculture due to their endosporeforming nature, important for industry, and their production of natural antimicrobial compounds(NACs) capable of antagonizing pathogens growth, biofilm formation and communication.

Objectives: Isolate and characterize different *Bacillus* spp. from the gut of aquaculture fish, capable of producing NACs antagonistic of fish bacterial diseases.

Methods: Heat-treated intestinal contents of *Sparus aurata*, *Diplodus sargus,* and *Dicentrarchus labrax* were used to obtain the gut sporeforming community. All isolates were screened for antimicrobial, anti-biofilm and anti-quorum-sensing activities, using established protocols. Significance of inhibition was evaluated by repeated measures ANOVA or 1-way ANOVA.

Results: A total of 176 isolates representing different colony morphologies and samples were obtained. Screening for NACs production revealed that 52% displayed antimicrobial activity. By characterizing the localization of the inhibitory molecules, the cell-free supernatants of three isolates (identified as *B.subtilis* by 16S rRNA sequencing), significantly (p<0.05) inhibited the growth and biofilm formation of several *Aeromonas*, *Vibrio*, *Photobacterium*, *Tenacibaculum*, *Edwardsiella* and *Shigella* species. Moreover, the three isolates produced compounds capable of interfering with acyl-homoserine-lactone signals, used in Gram- bacteria communication. These strains are being further studied to be used as future probiotics or source of bioactive molecules as tools to prevent aquaculture fish diseases.

PM013 The study of the lytic and curative potential of antimicrobial agents produced by Lysobacter capsici VKM-2533T

Alexey Afoshin[1], Irina Kudryakova[1], Nina Shishkova[2], Ilya Toropyguine[3], Michael Konstantinov[4], Alexandra Borovikova[5], Natalia Vasilyeva[1]

[1]PSCBR RAS G.K. Skryabin Institute of Biochemistry and Physiology of Microorganisms Russian Academy of Sciences, Pushchino, Moscow Region, Russian Federation
[2]State Research Center for Applied Microbiology and Biotechnology, Obolensk, Moscow Region, Russian Federation
[3]Institute of Biomedical Chemistry, Mocow, Russian Federation
[4]Pirogov Russian National Research Medical University, Moscow, Russian Federation
[5]Mari State University, Yoshkar-Ola, Russian Federation

Background: Widespread pathogenic microorganisms that are become more and more multi-resistant to all known antibiotics stimulate an intensive search and isolation from the environment of new efficient producers of antimicrobial agents. Representatives of the genus *Lysobacter* are known for their bacteriolytic properties. The focus of our research is *L.capsic* whose antimicrobial properties remain not fully understood studied yet.

Objectives: The study of the *L. capsici* lytic potential

Methods: Protein purification by FPLC, ultracentrifugation, MALDI-TOF, assays of bacteriolytic activity

Results: The ability of *L. capsici* to form vesicles was established. The vesicles demonstrated a wide spectrum of bacteriolytic activity against living opportunistic Gram-positive bacteria of the genera *Micrococcus, Bacillus, Corynebacterium* and *Staphylococcus*. It was found out that the culture liquid of *L. capsici* was efficient also against mycelial opportunistic fungi of the genera *Fusarium, Sclerotinia, Aspergilus,* as well as the yeasts *Saccharomyces* and *Candida*. It was of interest that the antifungal activity was localized in the vesicles.

For the first time, the β-lytic protease was purified from *L. capsici* culture liquid. The therapeutic effect of β-lytic protease was investigated in the case of staphylococcal sepsis (infection caused by *S. aureus* 55 MRSA) that was modeled in outbred white mice. It was found out that the intraperitoneal injection of β-lytic protease cleaned the surface of the mice kidneys from *Staphylococcus*.

The obtained results are promising for the further development of highly effective new generations of anti-staphylococcus drugs.

PM014 Identification of genes associated with beta-lactam resistance in clinical isolates of Gram-negative bacteria

Ricardo Oliveira[1;2;3], Andreia Azevedo[1;2;4;5], Nuno Azevedo[2], Carina Almeida[1;2;3]

[1]*CEB - Centre of Biological Engineering, University of Minho, Braga, Portugal*
[2]*LEPABE - Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering, University of Porto, Porto, Portugal*
[3]*INIAV, I.P. - National Institute for Agrarian and Veterinarian Research, Laboratory of Food Microbiology, Vairão, Vila do Conde, Portugal*
[4]*i3S - Instituto de Investigação e Inovação em Saúde, University of Porto, Porto, Portugal*
[5]*IPATIMUP - Institute of Molecular Pathology and Immunology, University of Porto, Porto, Portugal*

Background: Antibiotic resistance is rising to dangerous levels. New resistance mechanisms are reported every year, threatening the ability to control infectious diseases with available antimicrobial therapies. The characterization of the antibiotic resistance gene pool is therefore crutial for an accurate monitoring and control of high resistant bacteria.

Objectives: The objective of this study was to identify the genes associated with the production of carbapenemases, extended-spectrum β-lactamases (ESBL) and/or AmpC β-lactamases in a collection of gram-negative isolates from a central hospital in the northern region of Portugal.

Methods: Primers to amplify clinically relevant resistance genes were selected from the literature: *bla*KPC, *bla*IMP, *bla*VIM, *bla*OXA-48, *bla*OXA-23, *bla*NDM, *bla*SHV, *bla*TEM, *bla*CTX-M, *bla*CMY-2 and *bla*DHA. Two multiplex PCR sets were designed for the detection of carbapenemases, one for the ESBL and one for the AmpC β-lactamases. All assays were validated using 22 control strains containing characterized resistance genes. Subsequently, a set of 52 clinical isolates with antibiotic resistance were evaluated.

Results: PCR screening identified 13 (25.0%) isolates positives for carbapenemases genes. Of those, 4 (7.7%), including three *Klebsiella pneumoniae* and one *Escherichia coli*, were positive for *bla*KPC and 9 (17.3%) *Acinetobacter baumannii* were positive for *bla*OXA-23. ESBL genes were the most prevalent: 14 (26.9%) isolates were positive for *bla*SHV, 17 (32.7%) for *bla*CTX-M and 15 (28.8%) for *bla*TEM. For AmpC β-lactamases only 7 *Enterobacteriaceae* isolates were found positive for *bla*CMY-2 or *bla*DHA. Fourteen (26.9%) isolates did not contain any of the tested genes and 18 (34.6%) isolates contained two or more resistance genes.

PM015 Activity of Dodecameric Peptide Derived from Phage Peptide Library against Shigella flexneri and Salmonella enteritidis

Bit-Na Kim[1], Yang-Hoon Kim[2], Jiho Min[1]

[1]Chonbuk National University, Graduate School of Semiconductor and Chemical Engineering, Jeonju-si, Korea, Rep. of South
[2]Chungbuk National University, School of Biological Sciences, Cheongju, Korea, Rep. of South

Background: Microbial infections caused by bacteria have become a growing threat to public health worldwide. The excessive use of antibiotics to treat bacterial infection has led to the emergence of multidrug-resistant strains. Hence, the growing problem of microbial infections call for the development of new methods for the detection and specific inhibition of such pathogenic bacteria.

Objectives: To evaluate the potential activity of specific peptides associated with *Shigella flexneri* and *Salmonella enteritidis* which are the most prevalent bacterial foodborn pathogen for detection and growth inhibition.

Methods: We selected, from a Ph.D.-12 peptide library, peptides capable of specific binding to outer membrane protein of *S. flexneri* and *S. enteritidis*. The bacteria-binding properties of selected peptides to interact specifically with each strains were identified by using fluorescence image analysis. The specific inhibition activities of peptide were also estimated by measuring the growth curve of bacterial cells at OD 600 nm.

Results: After three rounds of panning, two specific peptides, which revealed common peptide (MHPNAGHGSLMR) and *Salmonella*-specific peptide (SSTLLNVVPKLH), were selected that exhibited higher binding affinities and specificity for *S. flexneri* and *S. enteritidis*. It was indirectly confirmed that each of the peptides adhered to the cell surface by immunofluorescence. Due to that, two peptides, common and *Salmonella*-specific, inhibited the growth of *S. flexneri* and *S. enteritidis*, respectively. This research was supported by Basic Science Research Program through the National Research Foundation of Korea(NRF) funded by the Ministry of Science, ICT & Future Planning(2017R1A2B4009775)

PM016 Saccharomyces Cerevisiae-Derived Lysosomes for Potential Application as Daunorubicin Delivery Vehicles for Acute Myeloid Leukemia (AML) Treatment

Wooil Choi[1], Mi-Young Heo[1], Yang-Hoon Kim[2], Jiho Min[1]

[1]Chonbuk National University, Graduate School of Semiconductor and Chemical Engineering, Jeonju-si, Korea, Rep. of South
[2]Chungbuk National University, School of Biological Sciences, Cheongju-si, Korea, Rep. of South

Background: The lysosomes derived from *Saccharomyces cerevisiae* are an intracellular organelle with an acidic lumen. Daunorubicin is widely used in the treatment of acute myeloid leukemia. Daunorubicin, weakly basic anticancer drug, is sequestered in the lysosomal compartment via a pH partitioning mechanism.

Objectives: We have studied how to encapsulate daunorubicin into lysosomes as drug delivery vehicles. We have optimized the time and concentration to encapsulate the drug. The retention time and the anticancer effect were evaluated. Therefore, lysosomal encapsulation method can be established, and it can lead to the application of the use of *S. cerevisiae*-derived lysosomes as drug delivery vehicles.

Methods: Recombinant *S. cerevisiae* harboring dominant-negative gene of YPT7 was constructed to control the size of lysosomes. The daunorubicin was loaded by mixing with lysosomes, depending on times and concentrations based on pH gradients. In addition, the loading efficiency and retention time of drugs on lysosomes were evaluated using fluorescence. Finally, daunorubicin encapsulated lysosomes were treated in HL60 cell line.

Results: We found that the size of the lysosomes was controlled. The appropriate time and concentration of daunorubicin for encapsulation on lysosomes were optimized with 3hrs and 40μg, respectively. In addition, we found that daunorubicin encapsulated lysosomes have shown the enhanced cytotoxicity against HL60. Therefore, in this study, it suggests that *S. cerevisiae*-derived lysosomes can be used as a drug delivery vehicle. This work was carried out with the support of "Cooperative Research Program for agriculture Science & Technology Development (Project No: PJ01267701)' Rural Development Administration, Republic of Korea.

PM017 Activity of Lysosomal-related Organelle Extract (LOE) from Saccharomyces cerevisiae to Reduce Melanin Color

Gyeongchan Jeon[1], Jihee Yoon[2], Jiho Min[2], Yang-Hoon Kim[3]

[1]Chonbuk National University, School of Semiconductor and Chemical Engineering, Jeonju, Korea, Rep. of South
[2]Chonbuk National University, Division of Chemical Engineering, Jeonju, Korea, Rep. of South
[3]Chungbuk National University, School of Biological sciences, Cheongju, Korea, Rep. of South

Background: Melanin is the most important factor to determine skin color. Currently developed skin whitening cosmetics have function for inhibiting melanin synthesis. However, these products take a long time to be effective and are not effective for hyperpigmentation. It was thought that the organelle which contain hydrolytic enzymes that can break down many kinds of biomolecules, lysosome can decompose produced melanin.

Objectives: we have tried to confirm the potential activity of lysosomal-related organelle extract (LOE) from *Saccharomyces cerevisiae* as novel materials for reducing color intensity of already produced melanin in skin. In addition, glutathione peroxidase (GPX) was selected to analyze the activity for melanin color reduction as a representative enzyme among LOE from *S. cerevisiae*.

Methods: The LOE was used to treat 100 ppm melanin solution, and the melanin residues treated with LOE were determined by absorbance at 450 nm. *S. cerevisiae* was constructed to highly express GPX tagged with green fluorescence protein.

Results: we found that the color intensity of melanin was reduced by LOE from *S. cerevisiae*. In addition, LOE via GPX overexpression was shown to enhance the activity of melanin color reduction. Therefore, peroxidase, which functioned to reduce melanin color, was mostly important to be maintained in LOE. These results provide insights into the regulation of LOE as new cosmetic materials to reduce the color intensity of melanin. This work was carried out with the support of "Cooperative Research Program for agriculture Science & Technology Development (Project No: PJ01267701)' Rural Development Administration, Republic of Korea.

PM018 Evaluation of bacterial endophytes isolated from aromatic rice cultivars as plant growth promoting agents for development of suitable biofertilizer

Anagha Krishnamoorthy[1], Mrinal Kumar Maiti[1]

[1]*Indian Institute of Technology Kharagpur, Department of Biotechnology, Kharagpur, India*

Background: The rampant usage of pesticides and chemical fertilizers to increase crop yield has led to severe environmental degeneration. To combat this synthetic chemical menace, sustainable agriculture must be employed. Therefore, exploring plant-microbe interactions could be an effective strategy for enhancing crop productivity. Endophytes are microorganisms that colonize within host plant tissues and aid in plant growth promotion (PGP) in different crops.

Objectives: The present study aims to address the following key questions in the domain of rice endophyte research: (i) How do endophytes influence the fitness of host plants? (ii) How do plants respond to single inoculations with endophytic bacterial strains upon seed priming with regard to PGP-characteristics?

Methods: Seed-borne endophytic bacteria were isolated from *in vitro*-grown calluses of selected aromatic rice cultivars Kalonunia and Dehradun Basmati, assayed for various PGP traits, and the PGP effect rendered by them on the host plant rice was investigated.

Results: Nine endophytic bacteria were isolated and identified through 16S *rRNA* gene sequence analysis. Out of nine endophytic bacteria, two *Pseudomonas* sp. exhibited all PGP traits tested. The endophytic *Pseudomonas* sp. PB002 and *Pseudomonas* sp. PB006 tested positive for nitrogen fixation, phosphate solubilization, phytohormone production and ACC deaminase activity. Further, single inoculations with these isolates on rice seeds revealed an increase in shoot length, fresh and dry weights, and chlorophyll content of the seedlings, in comparison to the uninoculated control set. Our findings indicate that the selected endophytic bacteria could be used for development of suitable biofertilizer consortia to improve rice crop productivity.

PM019 Cloning and characterization of an alcohol acetyltransferase gene GcAAT responsible for broad spectrum antifungal activity of endophytic Geotrichum candidum PF005

Mohor Mitra[1], Mrinal Kumar Maiti[1]

[1]*Indian Institute of Technology Kharagpur, Department of Biotechnology, Kharagpur, India*

Background: An endophytic yeast-like fungal strain of *Geotrichum candidum* PF005 isolated from eggplant fruit, was found to emit fruity scented antifungal volatile organic compounds (VOCs), primarily comprising of acetate esters. Alcohol acetyltransferases (AATs) are a group of enzymes that catalyze the formation of esters from alcohol and acetyl-CoA. However, these enzymes are not well characterized with regard to synthesis of antifungal compounds.

Objectives: The present study aims to explore the broad spectrum antifungal activity of *Geotrichum candidum* PF005 against stored grain pathogens and to evaluate the role of putative AAT from PF005 (GcAAT) in generation of antifungal VOCs.

Methods: Dual plate assay was conducted for evaluation of antifungal activity. Microscopy studies and TBARS assay were conducted to study the effect of VOCs on pathogen. *GcAAT* was cloned and characterized using southern hybridization, transcriptional profiling, heterologous expression in *AAT* knockout strain of *S. cereviciae* and confocal microscopy. Bioinformatics study was conducted for functional and structural predictions.

Results: PF005 was found to exhibit antifungal activity against ten out of twenty-two stored grain pathogens tested. The VOCs exhibited adverse effects on pathogen morphology, chitin distribution and generated oxidative stress in them. Ester precursor feeding resulted in increased transcript level of *GcAAT* in PF005. GcAAT was expressed in *AAT* knockout strain of *S. cereviciae*, which exhibited higher antifungal activity than untransformed control. Our results documented that GcAAT is a potentially important enzyme in antifungal ester production. Manipulation of GcAAT in heterologous or endogenous yeast system could be employed for mycofumigation of stored grain pathogens.

PM020 Effect of epigallocatechin gallate on gene expression of Staphylococcus aureus

Apisada Kitichalermkiat[1], Jun Sato[2], Takumi Sonoda[2], Yoshimitsu Masuda[1], Ken-ichi Honjoh[1], Takahisa Miyamoto[1]

[1]*Kyushu University, Department of Bioscience and Biotechnology, Fukuoka, Japan*
[2]*Kao Corporation, Tochigi, Japan*

Background: *Staphylococcus aureus* is one of the major foodborne pathogens that produces variety of extracellular toxins. Epigallocatechin gallate (EGCg), a type of polyphenol abundant in green tea, show the strong antibacterial activity against gram-positive bacteria.

Objectives: This study investigated the effect of EGCg on gene expression of *S. aureus* to clarify its mechanism of antibacterial action.

Methods: Microarray analysis was performed on *S. aureus* treated with and without 500 mg/L EGCg. The transcription changed genes by the treatment were identified and their changes in transcription level were confirmed by Real-Time qPCR. Moreover, the effects of EGCg on the function of genes with increased transcription was investigated by measuring gluconate uptake of *S. aureus*. The effect of EGCg on production of Staphylococcus enterotoxins were also investigated by measuring the amount of toxins using reversed passive latex agglutination (RPLA) method.

Results: Microarray analysis revealed that EGCg treatment of *S. aureus* resulted in increased and decreased transcription of 75 and 72 genes, respectively. Among them, genes related to the membrane transport which transcription increased more than 2-fold changes are *gntP*, *gntK*, *rumA*, *SAOUHSC_02723*, *SAOUHSC_01311*, and *SAOUHSC_02099*. The observation of gluconate uptake of EGCg-treated cells suggested that EGCg inhibited uptake of substrates for energy production. It seems that the cells treated with EGCg require more energy for recovery. Moreover, enterotoxin production was reduced in the presence of EGCg and completely inhibited at 500 mg/L. EGCg is one of the promising natural antibacterial agents since it inhibits growth of *S. aureus* and production of enterotoxin.

PM021 Genomics for the diagnosis, monitoring and the definition of remediation strategies of chlorinated hydrocarbon polluted sites

Charlotte Urien[1], Jennifer Hellal[2], Gwenaël Imfeld[3], Stéphane Vuilleumier[4], Catherine Joulian[2], Stephanie Ferreira[1]

[1]GenoScreen, Research and Development, LILLE, France
[2]BRGM, Geomicrobiology and Environmental Monitoring, Orléans Cedex 2, France
[3]University of Strasbourg/ENGEES, Laboratory of Hydrology and Geochemistry of Strasbourg (LHyGeS), Strasbourg Cedex, France
[4]University of Strasbourg, UMR 7156 CNRS, Strasbourg, France

Background: Chlorinated hydrocarbons are widespread contaminants in groundwater and subsurface ecosystems, thereby reflecting their massive use as industrial solvents in the last decades. Their hydrophobicity has led to their accumulation and persistence in aquifers, while their toxicity and potential carcinogenicity pose serious risks to human health and environment. However, microbial chlorinated hydrocarbon degradation can occur naturally *in situ*.

Objectives: The project BioDissPOL (microbial biomarkers: applicability for diagnosis and monitoring of polluted sites) addresses how dissipation of chlorinated hydrocarbons is linked with microbial community composition and relative abundance of selected functional genes, with the aim to develop a widely accepted framework to monitor bioremediation at contaminated sites.

Methods: We collected and analyzed groundwater from 12 piezometers at a multi-polluted site in France, at the source, in the plume and its fringes. Taxonomical and functional microbial community profiles were obtained from analysis of extracted DNA by shotgun and amplicon metagenomics approaches (Illumina MiSeq). In parallel, we developed a dedicated reference database of functional genes involved in biodegradation of chlorinated hydrocarbons.

Results: Results show that functional gene frequencies depend on piezometer origin with highest level in the plume and minus level in the fringes. Those results fit with expected bioremediation pattern by natural microbial communities. They also highlight that evaluation of chloroethene biodegradation using DNA-based approaches provides valuable information, such as functional genes distribution and taxonomical composition of the bacterial communities, for implementation of bioremediation strategies at contaminated industrial sites.

PM022 Synthesis and evaluation of aromatic surfactants as potential antibacterial and cytotoxic agents

Kenia Barrantes[1], Mary Fuentes[2], Luz Chacon[1], Rosario Achi[1], Jorge Granados-Zuñiga[3], María José Alvarado[4], Luis Somarribas[3], José Vázquez-Tato[5], M.Pilar Vázquez-Tato[5], Julio Seijas[5], Victor Soto-Tellini[4]

[1]*Health Research Institute, Nutrition Infection Section, Costa Rica*
[2]*The University of Texas at El Paso, El Paso, United States*
[3]*School of Medicine, Biochemistry, Costa Rica*
[4]*School of Chemistry, Organic Chemistry, Costa Rica*
[5]*University of Santiago de Compostela, Facultad de Ciencias, Santiago de Compostela, Spain*

Background: Antimicrobial resistance is a public health problem. Drug resistance is a common phenomenon that occurs in the treatment of cancer. There is a concerted global effort to identify new molecules with antibacterial and/or antitumor properties.

Objectives: aim of this study was to describe the synthesis of three novel aromatic surfactants: C8, C18, and NOBA. We then evaluated their antimicrobial and cytotoxic activities.

Methods: Ether formation was performed via Williamson syntheses. Antimicrobial activity was evaluated with a broth culture dilution method according to CLSI. Bacterial strains were: *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 6538, and *Pseudomonas aeruginosa* ATCC 14502. *Shigella flexneri* INISA 01 was also used and was nosocomial and resistant to multiple antibiotics. The hemolytic activity in red blood cells was assessed with the hemoglobin release assay according to Palermo and Kuroda.

The cytotoxicity was assessed according to Fronza et al. (2011) with modifications. Three tumor cell lines were used to test the cytotoxic activity of the new compounds: HeLa (human cervix carcinoma), NCH82 (human glioblastoma), and MCF7 (human breast adenocarcinoma). Vero (green monkey kidney) and L929 (mouse fibroblast) lines were used as normal cell controls.

Results: The three aromatic derivatives showed antibacterial activity against one of the four microorganisms. The effects of C8 and NOBA were observed in HeLa cells (human cervix carcinoma). Other controls include Vero (green monkey kidney cell line) and L929 (mouse fibroblast cell line) as controls of normal cell lines; cytotoxic effects were observed with an IC50 value higher than CPT.

PM023 The effect of yeast cultures on the microbial composition and fermented cocoa volatile compounds

Jatziri Mota Gutierrez[1], Cristian Botta[1], Ilario Ferrocino[1], Manuela Giordano[1], Luca Cocolin[1]

[1]*University of Turin, Department of Agricultural, Forestry and Food Science, Grugliasco, Italy*

Background: Starter cultures are generally used to control microbial communities and thus, aromatic profiles. The purpose of this study is to provide new insights into the development of microbial volatile compounds (mVOCs) formed at the beginning of the cocoa fermentation and proposed a correlation analysis to understand their interactions with microbial communities.

Objectives: Therefore, the aim of the present work is to assess the effect of yeast starter cultures used on two different type of fermentations (box and heap) on the microbial composition and mVOCs.

Methods: In this study, we performed a chemical, microbiological and targeting analysis sequences on fermented Forastero hybrid cocoa beans originated from Cameroon. This cocoa mass was inoculated with *Saccharomyces cerevisiae* (ID67) and a mixed culture of *S. cerevisiae* and *Torulaspora delbrueckii* strain (ID103) in a radio 1:1 at 10^7 CFU g^{-1}. In addition, spontaneous fermentations were also carried out as control.

Results: Our results showed that yeast cultures modulated the microbial ecology, especially *Hanseniaspora, Lactobacillus plantarum, Lactobacillus fermentum* and *Acetobacter pasteurianus at* the beginning of the cocoa fermentation and the volatilome profile. However, this effect is dependent on the type of fermentation used and environmental conditions. In addition, the correlation dataset showed a significant different association between mVOCs and mycobiota, this information confirms the complexity of the aroma production by microbial communities in fermented cocoa beans. Special attention is paid to find a suitable starter culture for fermented cocoa beans to produce more aromatic chocolate.

PM024 PilVax- A novel peptide carrier for the development of vaccines against tuberculosis

Sam Blanchett[1], Jacelyn Loh[1], Thomas Proft[1]

[1]*University of Auckland, Molecular Medicine and Pathology, Auckland, New Zealand*

Background: PilVax is a peptide delivery strategy for the generation of highly specific mucosal immune responses. The food-grade bacterium *Lactococcus lactis* is used to express selected peptides engineered within the Group A Streptococcal pilus, allowing for peptide amplification, stabilisation, and enhanced immunogenicity.

Objectives: The present study aims to demonstrate the suitability of PilVax for the generation of novel peptide vaccines against tuberculosis caused by the bacterium *Mycobacterium tuberculosis (M. tuberculosis)*.

Methods: Selected peptides (B cell and T cell epitopes), derived from tuberculosis vaccine targets ESAT-6 and Ag85B, were genetically engineered into loop regions of the pilus backbone subunit and expressed in *L. lactis.* Western blots and flow cytometry confirmed pilus formation on *L. lactis*. Mice were vaccinated with the PilVax-Ag85B construct and the B cell responses analysed by ELISA while T cell responses were analysed by flow cytometry.

Results: Vaccination with PilVax-Ag85B resulted in peptide-specific humoral and cellular responses. High serum antibody titres of IgG and IgA show the ability to produce antibodies against the cognate peptide. However, it remains to be seen whether these antibodies are protective.

Significant Ag85B peptide specific CD4+ T cell responses were observed that were similar to those seen in BCG immunised mice. We are currently investigating humoral, cellular and cytokine responses in mice vaccinated with PilVax-Ag85B and three PilVax-ESAT-6 constructs (each expressing a different peptide). Future studies include challenge of PilVax-vaccinated mice with *M. tuberculosis*.

PM025 Proteomic investigation of the light induced stress response in the purple non sulphur bacterium Rhodospirillum rubrum

Guillaume Bayon-Vicente [1], rob onderwater[2], ruddy wattiez[1], baptiste leroy[1]

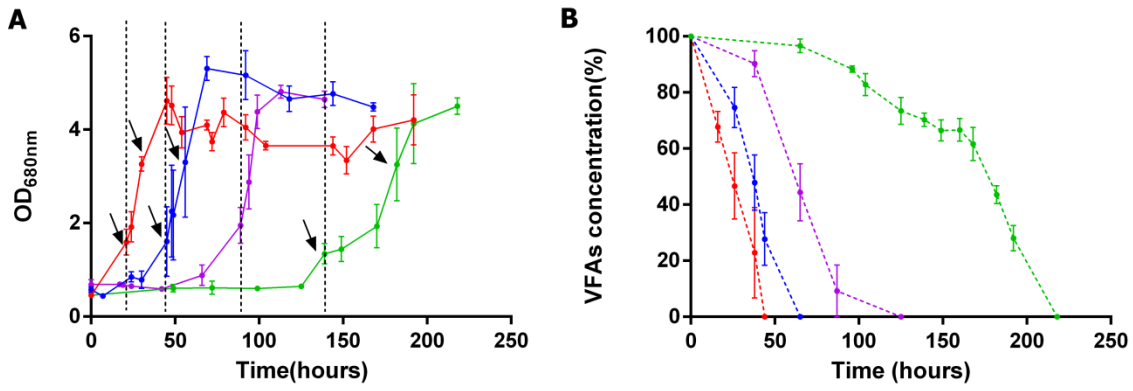
[1]*University of Mons, Proteomic and Microbiology, Mons, Belgium*
[2]*materia nova, ath, Belgium*

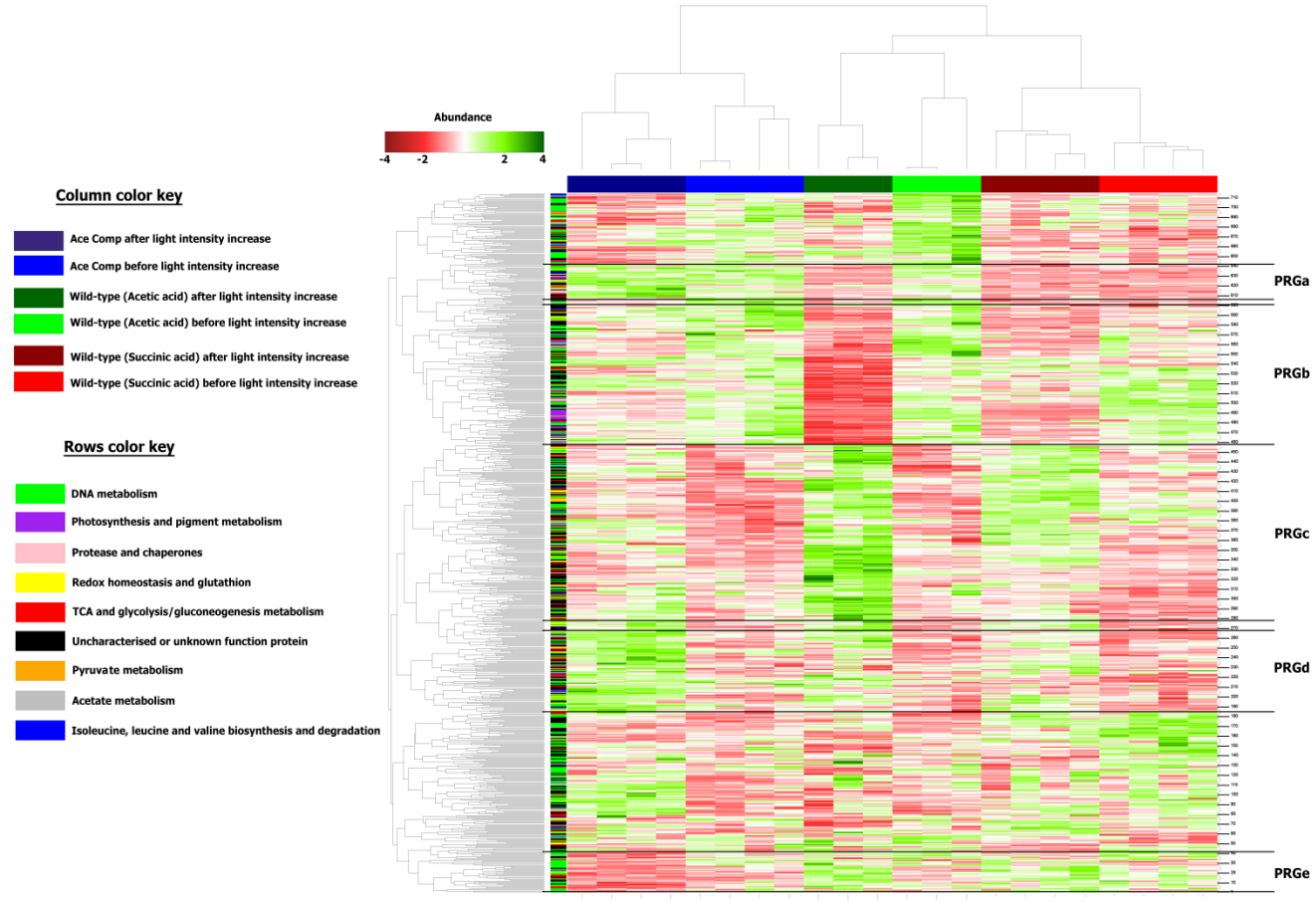
Background: Our lab investigated the acclimatisation occurring after multiple cultivations of *Rs.* rubrum, a Purple Non-Sulphur Bacterium (PNSB) assimilating a broad range of carbon sources including Volatile Fatty Acids (VFAs), on acetate characterised by the amplification of a 60-kb genome fragment containing genes coding for key enzymes of the ethylmalonyl-CoA pathway. In addition, due to the reduced lag phase, this adapted strain, named "acetate competent strain", is of great interest for biotechnological processes.

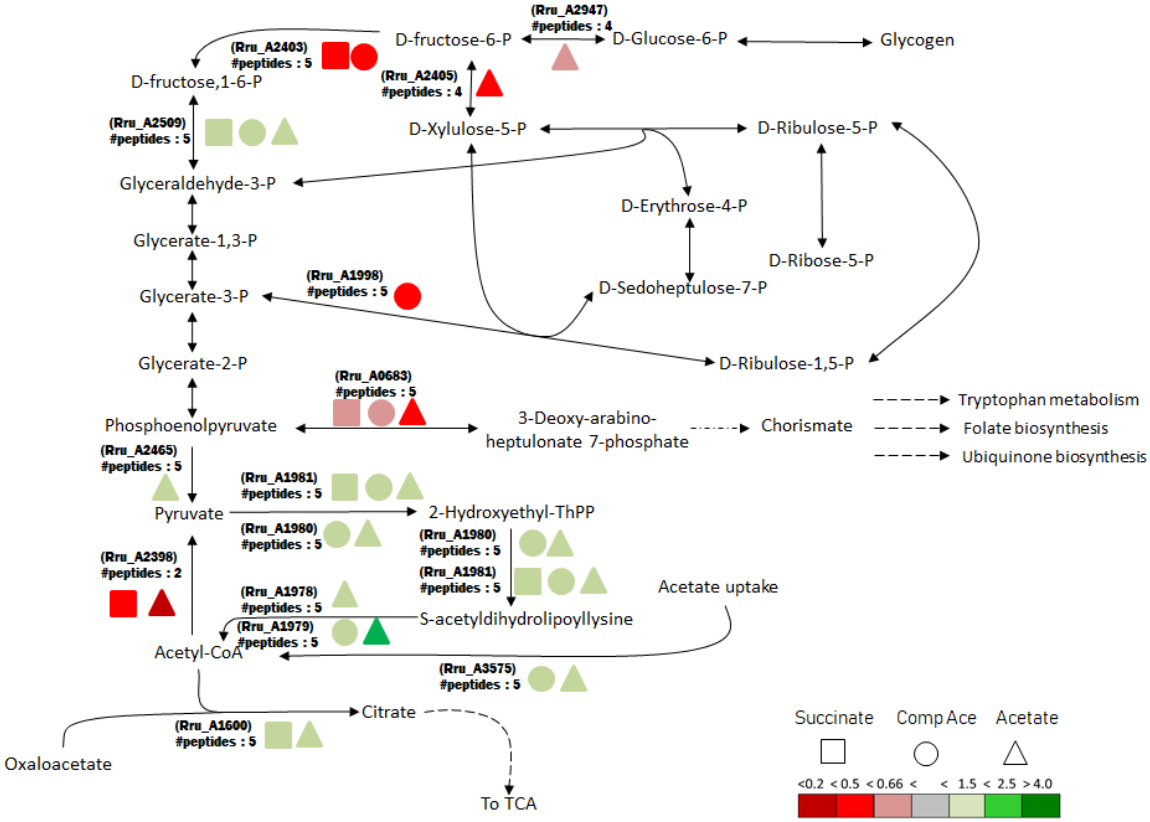
Objectives: We attempt to fully characterise this new strain and to deeply understand the link between EMC pathway, the reduced lag phase and the HCO_3^- dependence of *Rhodospirillum rubrum* growth in presence of acetic acid

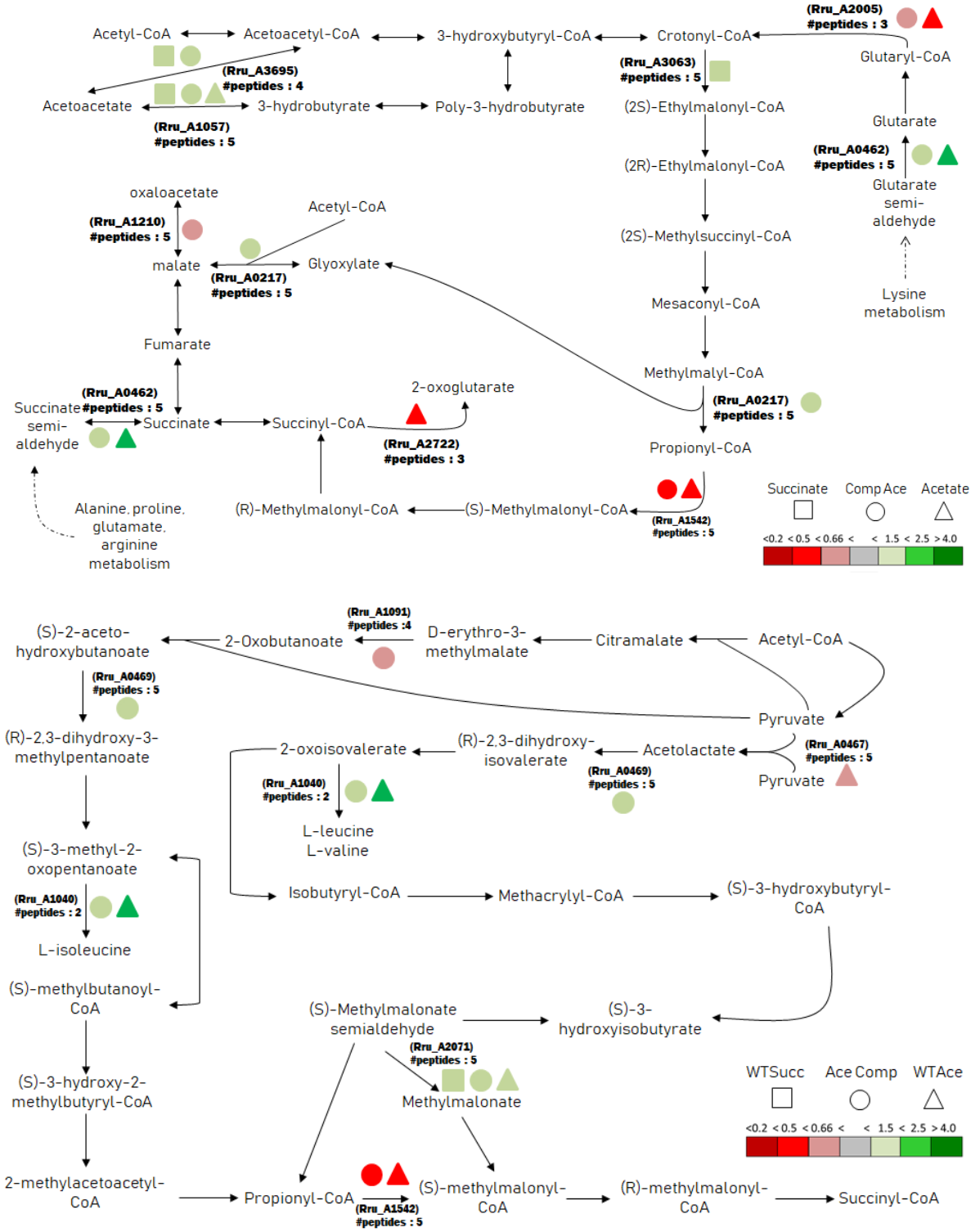
Methods: *Rs. Rubrum* WT and the new acetate competent strain were cultivated in presence of acetic acid and the light intensity was switched from 50 to 150 µmol of photon/m² s. We used unlabelled relative quantification comparative proteomic analyses as well as bioinformatic tools such as protein theoritical network and heatmap to first investigate *Rs. Rubrum* response to this new parameter.

Results: Data demonstrated that high light intensity deeply impact the photosynthesis and pigment metabolism as well as the redox homeostasis indicating that light intensity increase has induced an intracellular redox stress. Furthermore, the light regime also influences the central carbon metabolism of *Rhodospirillum rubrum*. Whereas the response of the wild type strain is mainly characterised by the higher abundance of thioredoxin, glutathione and other detoxification proteins, the acetate competent strain showed outstanding resistance indicating the EMC pathway constitutes an electron sink.




Abundance
-4 -2 2 4
Column color key
Ace Comp after light intensity increase
Ace Comp before light intensity increase
Wild-type (Acetic acid) after light intensity increase
Wild-type (Acetic acid) before light intensity increase
Wild-type (Succinic acid) after light intensity increase
Wild-type (Succinic acid) before light intensity increase
Rows color key
DNA metabolism
Photosynthesis and pigment metabolism
Protease and chaperones
Redox homeostasis and glutathion
TCA and glycolysis/gluconeogenesis metabolism
Uncharacterised or unknown function protein
Pyruvate metabolism
Acetate metabolism
Isoleucine, leucine and valine biosynthesis and degradation
PRGa
PRGb
PRGc
PRGd
PRGe


(Rru_A2947) #peptides : 4
D-fructose-6-P
D-Glucose-6-P
Glycogen
(Rru_A2403) #peptides : 5
(Rru_A2405) #peptides : 4
D-fructose,1-6-P
(Rru_A2509) #peptides : 5
D-Xylulose-5-P
D-Ribulose-5-P
Glyceraldehyde-3-P
D-Erythrose-4-P
Glycerate-1,3-P
D-Ribose-5-P
(Rru_A1998) #peptides : 5
Glycerate-3-P
D-Sedoheptulose-7-P
Glycerate-2-P
D-Ribulose-1,5-P
(Rru_A0683) #peptides : 5
Phosphoenolpyruvate
3-Deoxy-arabino-heptulonate 7-phosphate
Chorismate
Tryptophan metabolism
Folate biosynthesis
Ubiquinone biosynthesis
(Rru_A2465) #peptides : 5
(Rru_A1981) #peptides : 5
Pyruvate
2-Hydroxyethyl-ThPP
(Rru_A1980) #peptides : 5
(Rru_A1980) #peptides : 5
(Rru_A2398) #peptides : 2
(Rru_A1981) #peptides : 5
Acetate uptake
(Rru_A1978) #peptides : 5
S-acetyldihydrolipoyllysine
(Rru_A1979) #peptides : 5
Acetyl-CoA
(Rru_A3575) #peptides : 5
Citrate
(Rru_A1600) #peptides : 5
Oxaloacetate
To TCA
Succinate
Comp Ace
Acetate
<0.2 < 0.5 < 0.66 < < 1.5 < 2.5 > 4.0


Acetyl-CoA
Acetoacetyl-CoA
3-hydroxybutyryl-CoA
Crotonyl-CoA
(Rru_A2005)
#peptides : 3
Glutaryl-CoA
(Rru_A3695)
#peptides : 4
Acetoacetate
3-hydrobutyrate
Poly-3-hydrobutyrate
(Rru_A3063)
#peptides : 5
(2S)-Ethylmalonyl-CoA
(Rru_A1057)
#peptides : 5
Glutarate
(Rru_A0462)
#peptides : 5
(2R)-Ethylmalonyl-CoA
Glutarate
semi-
aldehyde
oxaloacetate
Acetyl-CoA
(Rru_A1210)
#peptides : 5
(2S)-Methylsuccinyl-CoA
malate
Glyoxylate
(Rru_A0217)
#peptides : 5
Lysine
metabolism
Mesaconyl-CoA
Fumarate
Methylmalyl-CoA
2-oxoglutarate
(Rru_A0462)
#peptides : 5
(Rru_A0217)
#peptides : 5
Succinate
semi-
aldehyde
Succinate
Succinyl-CoA
(Rru_A2722)
#peptides : 3
Propionyl-CoA
Succinate
Comp Ace
Acetate
<0.2 < 0.5 < 0.66 < < 1.5 < 2.5 > 4.0
Alanine, proline,
glutamate,
arginine
metabolism
(R)-Methylmalonyl-CoA
(S)-Methylmalonyl-CoA
(Rru_A1542)
#peptides : 5
(Rru_A1091)
#peptides :4
(S)-2-aceto-
hydroxybutanoate
2-Oxobutanoate
D-erythro-3-
methylmalate
Citramalate
Acetyl-CoA
(Rru_A0469)
#peptides : 5
Pyruvate
(R)-2,3-dihydroxy-3-
methylpentanoate
2-oxoisovalerate
(R)-2,3-dihydroxy-
isovalerate
Acetolactate
(Rru_A0467)
#peptides : 5
(Rru_A0469)
#peptides : 5
Pyruvate
(Rru_A1040)
#peptides : 2
L-leucine
L-valine
(S)-3-methyl-2-
oxopentanoate
Isobutyryl-CoA
Methacrylyl-CoA
(S)-3-hydroxybutyryl-
CoA
(Rru_A1040)
#peptides : 2
L-isoleucine
(S)-methylbutanoyl-
CoA
(S)-Methylmalonate
semialdehyde
(S)-3-
hydroxyisobutyrate
(Rru_A2071)
#peptides : 5
WTSucc
Ace Comp
WTAce
<0.2 < 0.5 < 0.66 < < 1.5 < 2.5 > 4.0
(S)-3-hydroxy-2-
methylbutyryl-CoA
Methylmalonate
2-methylacetoacetyl-
CoA
Propionyl-CoA
(Rru_A1542)
#peptides : 5
(S)-methylmalonyl-
CoA
(R)-methylmalonyl-
CoA
Succinyl-CoA

PM026 Development of self-healing biocement: preliminary studies

Christine Spencer[1], Henrik Sass[2]

[1]*Cardiff University, School of Engineering, Cardiff, United Kingdom*
[2]*Cardiff University, School of Earth and Ocean Sciences, United Kingdom*

Background: Microbially induced calcium carbonate precipitation (MICP) has been attracting growing interest in respect of its potential use for ground improvement by the process of biocementation. Recent studies have demonstrated the potential of MICP to enable self-healing, through the injection of nutrients and precursor chemicals into degraded biocement.

Objectives: The focus of this three-year research project will be to develop a truly autonomous self-healing process, by facilitating the supply of nutrients and precursor chemicals from within the biocement matrix, to improve the durability and sustainability of geotechnical structures.

Methods: The potential of carrier materials for the effective immobilisation and release of the nutrients and precursor chemicals required for MICP has been explored. A preliminary study has subsequently been undertaken utilising expanded perlite, within a biocement produced using Sporosarcina ureae. Calcium production during the biocementation process has been determined using ICP-OES.

Results: This paper presents the results from preliminary investigations. Results obtained demonstrate that diatomaceous earth, expanded perlite and natural fibres such as jute have the potential to be utilised for the immobilisation and supply of the required nutrients and precursor chemicals to enable MICP. This combined with the proven spore forming ability of Sporosarcina ureae, indicates that in principal autonomous self-healing of biocement can be achieved.

PM027 Study of fungal biodegradability of an innovative solid-solid phase change material for thermal energy storage

Plancher Lionel[1], Nguyen Giao[2], Ledesert Béatrice[3], Melinge Yannick[4], Hebert Ronan[3], Patrick Di Martino[5]

[1]*Université de Cergy-Pontoise*
[2]*Université de Cergy-Pontoise, Laboratoire LPPI*
[3]*Université de Cergy-Pontoise, Laboratoire GEC*
[4]*Université de Cergy-Pontoise, Laboratoire L2MGC*
[5]*Université de Cergy-Pontoise, Laboratoire ERRMECe, Neuville sur Oise, France*

Background: Fungal development inside buildings can cause deterioration of building materials and affect the health of building occupants. Fungi can acquire nutrients using extracellular enzymes by breaking down natural or artificial complex polymers. Therefore, when developing a new polymer-based building material, it is important to study its fungal biodegradability.

Objectives: Phase change materials (PCMs) are used to passively regulate the interior temperature of buildings. The solid-solid PCMs consist of a cross-linked semi-crystalline polymer which undergoes a crystalline-amorphous thermochemical transition while maintaining its solid state. In the present study, the fungal biodegradability of an innovative solid-solid PCM based on polyurethane was tested.

Methods: The powdered MCP was introduced into supercooled agar media, the whole being then poured into a round petri dish. Four types of media were used: agar 1.5%, agar 1.5% + MCPs 5%, agar 1.5% + malt extract agar 0.5%, agar 1.5% + malt extract agar 0.5% + MCPs 5%. A calibrated fragment of mycelium from 5 days fungal preculture was deposited in the centre of each test culture medium. Fungal growth at 23 ° C was monitored by measuring fungal mycelium radius over a 23 days-period. Six mould strains belonging to the genera *Aspergillus*, *Penicillium*, *Cladosporium*, *Trichoderma*, *Aureobasidium*, and *Mucor* were tested.

Results: The MCP tested had no impact on the growth of *Aspergillus*, *Trichoderma*, *Aureobasidium*, and *Mucor*. The growth of *Penicillium* and *Cladosporium* was increased in the presence of MCP. Infrared spectroscopy analysis is in progress to study the biodegradation process of this MCP.

PM028 Use of Fluorescence Foldscope as an effective tool for detection of biofilm formation in Pseudomonas aeruginosa

Chandrayee Deshamukhya[1], Amitabha Bhattacharjee[1], Bhaskar Jyoti Das[1], Debadatta Dhar Chanda[2], Atanu Chakravarty[2]

[1]Assam University, Department of Microbiology, Silchar, India
[2]Silchar Medical College and Hospital, Department of Microbiology, India

Background: *Pseudomonas aeruginosa,* an opportunistic pathogenic bacterium has biofilm forming ability, and can evade immune response and antimicrobial chemotherapy. There are several methods available for detecting biofilms which require sophisticated instrumentation and expertise. The present study used an improvised device, "fluorescence foldscope"-an origami based fluorescence microscope as an easy and effective tool to detect biofilm formation.

Objectives: To detect biofilm formation in clinical isolates of *Pseudomonas aeruginosa* using fluorescence foldscope.

Methods: Three representatives of *Pseudomonas aeruginosa* of clinical origin CD1,CD2 and CD3(carrying no *bla* genes, $bla_{\text{CTX-M-15}}$, $bla_{\text{NDM-1}}$ respectively) and reference strains PA01 and ATCC27853 were taken for the study; cultured in LB broth without and with carbapenem & cephalosporin; diluted to 1:100 in LB;seeded with sterile glass slides at 90° angle and incubated for 5 consecutive days. The foldscope was improvised to detect fluorescence with the help of filter(excitation and emission) and blue LED and the slides were observed under it. The biofilm forming capacity was further validated with conventional methods.

Results: CD1 and CD2 formed biofilm as they emitted significant amount of fluorescence both at normal and subinhibitory carbapenem and cephalosporin pressure, maximum fluorescence being observed between 48 and 72 hours respectively, whereas no significant fluorescence was observed in CD3. The results were supported by conventional methods. The study came up with a very simple paper based microscopic method which can detect biofilm formation. Fluorescence foldscope, hence, will prove to be a potential cost effective and reliable tool for early diagnosis in clinical settings with limited resources.

PM029 Novel taxonomy-independent deep learning microbiome approach allows for accurate classification of different forensically relevant human epithelial materials

Celia Díez López[1], athina vidaki[1], arwin ralf[1], diego montiel gonzález[1], djawad radjabzadeh[2], robert kraaij[2], andré g uitterlinden[2], cordula haas[3], oscar lao[4], manfred kayser[1]

[1]erasmus mc medical center rotterdam, genetic identification, rotterdam, Netherlands
[2]erasmus mc medical center rotterdam, internal medicine, rotterdam, Netherlands
[3]zurich institute of forensic medicine, zurich institute of forensic medicine, zürich, Switzerland
[4]centre for genomic regulation, population genomics, barcelona, Spain

Background: Correct identification of human epithelial materials such as from skin, saliva and vaginal origin is relevant in forensic casework, providing crucial information for crime reconstruction. However, their overlap in human cell type composition provides challenges when using human cell biomarkers, while the microbiota composition of these three tissue types largely differs.

Objectives: We introduce a novel taxonomy-independent deep learning microbiome approach for tissue-type classification of human epithelial materials.

Methods: 50 taxonomy-independent deep learning networks were trained using validated 16S rRNA gene sequencing data from the Human Microbiome Project of 1,636 skin, oral and vaginal samples to classify these three tissues. Validation testing was performed in newly-generated 16S rRNA gene sequencing data using the Ion Torrent™ PGM from 110 test samples: 56 hand skin, 31 saliva and 23 vaginal specimens. Forensically-oriented validation testing was performed in mock casework samples, 19 freshly-prepared and 22 samples aged for 1 up to 7.6 years.

Results: Body-site classification accuracy was high, with AUC values of 0.989 for skin, 0.998 for oral, and 0.988 for vaginal secretion. Misclassifications were limited to 1 (2%) skin and 1 (4%) vaginal sample, while 3 (5%) skin samples showed inclusive results based on a probability threshold of 0.7. All of the 19 fresh and 17 (77%) of the 22 aged mock casework samples were correctly tissue-type classified. Comparing the microbiome results with previous human mRNA-based tissue identification testing in the same 16 aged mock casework samples reveals that approach performs better or equally in the majority of the samples.

PM030 Biodegradation of a polyether-polyurethane-acrylic coating by the microbial community bp8

Itzel Gaytán[1], Manuel Burelo[2], Martín Vargas-Suárez[3], Javier Cruz-Gómez[4], Herminia Loza-Tavera[5]

[1]Facultad de Química-Universidad Nacional Autónoma de México, Bioquímica, Ciudad de México, Mexico
[2]Instituto de Investigaciones en Materiales-Universidad Nacional Autónoma de México, Polímeros , Ciudad de México , Mexico
[1]Facultad de Química-Universidad Nacional Autónoma de México, Bioquímica, Ciudad de México , Mexico
[4]Facultad de Química-Universidad Nacional Autónoma de México, Ingeniería Química , Ciudad de México, Mexico
[5]Facultad de Química-Universidad Nacional Autónoma de México, Bioquímica, Ciudad de México, Mexico

Background: Polyurethanes (PU) are plastic polymers widely used in modern life. However, the large use, high durability and lack of effective recycling processes cause major environmental problems. Hence, biodegradation is an eco-friendly solution to reduce this problem.

Objectives: To describe the ability of the microbial community BP8 for degrading a polyether-polyurethane-acrylic (PE-PU-A) coating that also has xenobiotic additives, some of them containing the recalcitrant ether bond

Methods: BP8 community was isolated from a landfill. PE-PU-A coating was supplemented as the only carbon source in mineral medium. Growth was measured as dry-weight and carbon consumption by elemental analyses. The MATH method and SEM were used to observe cell-substrate interactions. FTIR, TGA, DSC, and GPC analyses determined the physicochemical modifications generated by BP8 to the polymer, and by GC-MS analysis additives were quantified and PE-PU-A breakdown products were identified

Results: BP8 was able to degrade the xenobiotics and the PE-PU-A polymers present in the coating. BP8 consumed 50% of the carbon source during the 20-days of culture and showed diauxic growth suggesting that more than one coating component is metabolized. At early times, BP8 metabolized all the coating additives. Polymer biodegradation showed by decrease in molecular size, breaking of the soft and hard domains, and by the chemical modifications in ester, ether, urethane, and aromatic groups was mediated by cells-substrate interactions. In the analysis of PE-PU-A breakdown products, organic acids, alcohols, esters, ethers, alkanes, amines, and aromatic compounds were observed, indicating that PE-PU-A biodegradation occurs by hydrolytic and oxidative mechanisms.

PM031 Mechanistic insights into inactivation of Mycobacterium by atmospheric cold plasma

Dana Ziuzina[1], Carolina Mehaffy[2], Karen Dobos[2], Paula Bourke[1]

[1]*Technological University Dublin, School of Food Science and Environmental Health, Dublin, Ireland*
[2]*Colorado State University, Department of Microbiology, Immunology & Pathology, United States*

Background: Atmospheric cold plasma (ACP) is increasingly investigated as an alternative sterilisation technology, which uses reactive oxygen and nitrogen species that are effective against wide range of environmental, medical device associated and foodborne pathogenic microorganisms. The genus *Micobacterium* contains species that cause significant morbidity and mortality worldwide. *Micobacterium* spp. posses innate resistance to biocides, limiting the potency of available disinfectants thereby impacting infection control.

Objectives: To study the effect of cold plasma generated in a gaseous phase, plasma activated water (PAW) and the potential of the combined treatment effect on viability of *M. smegmatis* in liquid or in a concentrated form.

Methods: *M. smegmatis* cells suspended either in phosphate buffer solution (PBS), in PAW or in a form of a cell pellet were treated with plasma gas (PG), generated on high voltage dielectric barrier discharge atmospheric air enclosed plasma system. The viability of *M. smegmatis* was determined by using plate count and metabolic activity assays. A range of bioassays were performed to elucidate the mechanism of action of plasma on *Mycobacterium* cells.

Results: PG effectively reduced populations of pelleted cells with up to ~9 $\log_{10}$ CFU/ml achieved after 1 min of treatment. Lower inactivation levels were obtained for cells suspended in PBS. Combining PG and PAW, bacterial populations were reduced by ~8 $\log_{10}$ CFU/ml after 10 min of treatment, comparing to ~1.4 $\log_{10}$ CFU/ml obtained in PBS. Spectrophotometric and fluorescent based techniques allowed understanding mechanisms underlying changes in cellular components in reponse to each plasma treatment approach.

PM032 Cocktail of three bacteriocinogenic Escherichia coli isolates inhibits colibacteriosis in piglets

Matěj Hrala[1], Juraj Bosák[1], Ivana Kolackova[2], Renata Karpiskova[2], David Smajs[1]

[1]*Masaryk University, Faculty of Medicine, Department of Biology, Brno, Czech Republic*
[2]*Veterinary Research Institute, Department of Bacteriology, Brno, Czech Republic*

Background: Infections caused by pathogenic *Escherichia coli* strains (colibacteriosis) are responsible for weight loss and mortality of pigs. The numbers of colibacteriosis cases rise worldwide and these infections are often caused by multi-resistant *E. coli* strains. Bacteriocins are antimicrobial substances produced by bacteria able to kill other bacteria. Bacteriocins are successfully used in food preservation, in veterinary medicine and have a promising potential also in human medicine.

Objectives: Identification and characterization of bacteriocinogenic *E. coli* strains and analysis of their therapeutic potential against pathogenic *E. coli* strains using piglet model.

Methods: Alltogether, 2,643 collected human and animal *E. coli* isolates were tested for virulence (18 virulence determinants) and bacteriocinogeny (30 bacteriocin determinants) using PCR detection. Out of them, three nonpathogenic and bacteriocinogenic strains (*E. coli* 582, *E. coli* B771, and *E. coli* B1172) were tested against common etiologic agents of swine colibacteriosis (enterotoxigenic *E. coli*; ETEC). The cocktail of three *E. coli* strains was co-cultivated with common ETEC types (F4 and F18) *in vitro*, and also tested against experimental ETEC infection in piglets. The numbers of shed pathogens and clinical manifestation of colibacteriosis were observed during experimental infection.

Results: The cocktail of bacteriocinogenic strains (producing 8 various bacteriocins) completly eliminated pathogens in 48 hours during *in vitro* co-cultivations. *In vivo*, the aplication of cocktail to experimental piglets resulted in decreased pathogen-shedding and shorter period ($p<0.01$) and lower severity of diarhea ($p<0.05$). Obtained results indicate therapeutic potential of used bacteriocinogenic cocktail in the prevention and treatment of colibacteriosis of post-weaning piglets.

PM033 Biosensors for pathogen detection

Juan Leva[1], Paul Millner[1], Sally Peyman[2]

[1]*University of Leeds, Scool of Biomedical sciences, Leeds, United Kingdom*
[2]*E C Stoner Building, Molecular & Nanoscale Physics Research Group, Leeds, United Kingdom*

Background: Evolution of pathogens along with human beings is a burden, and resistance to antibiotics and antivirals is becoming widespread. Therefore, early detection of these microorganisms in humans or animals is essential to provide efficient and adequate treatment. Electrochemical biosensors for pathogen detection have emerged in the last decade in response to the current methodologies for diagnosis which are expensive, labour-intensive, require skilled workers and often slow. *Escherichia coli*, *Acinetobacter baumannii* and *Klebsiella pneumoniae* are tested using electrochemical impedance spectroscopy (EIS) following two different approaches: an antibody-antigen based method and a novel adhesin-extracelular matrix proteins (ECM) based method.

Objectives: The aim is to detect and quantify these bacteria comparing two different biosensor constructs. In the first format, specific antibodies against bacteria are tested. Whilst on the second approach, ECM proteins will be used to adhere to adhesins on the pathogen surface. Both detection modes will be assessed and compared.

Methods: Gold screen printed electrodes are used as a transducer platform. Depending on the type of detection, the bioreceptors consist of antibodies or ECM proteins and different concentrations of analyte are tested and sensor interrogated by EIS. The impedance values are correlated with pathogen concentration.

Results: First experiments using a typical immunosensor for detection of bacteria have been successful and correlate well with bacterial concentration. Adhesin-ECM protein based methodologies will be performed and then compared. The best type of biosensor will be inserted into lab-on-a-chip microfluidic device.

PM034 Single-cell characterization of bacterial membrane properties with an optical microcavity

Manon Tardif[1], Rita Therisod[2], Emmanuel Picard[3], Pierre R. Marcoux[4], Victor Gaude[1], Jean-Baptiste Jager[3], Romuald Houdré[2], Emmanuel Hadji[3], David Peyrade[1]

[1]LTM CNRS, Laboratoire des technologies de le Microélectronique, Grenoble, France
[2]EPFL, Institut de Physique, Lausanne, Switzerland
[3]CEA Grenoble Center, Inac, Institut Nanosciences et Cryogénie, Grenoble, France
[4]Cea Leti, Division microtechnologies for biology and healthcare, Grenoble, France

Background: Many tests in microbiology require large biomasses, that is why clinical microbiology has relied on culture as the standard requirement before characterizing pathogens. But this culture is time-consuming. In this context, single-cell monitoring would be a significant breakthrough compared to Petri dishes culture.

Objectives: A first step was achieved by the demonstration of single bacterium trapping by optical tweezers and integrated photonics. Here, the nondestructive real-time state monitoring of a single alive trapped bacterium is demonstrated.

Methods: In order to achieve this, a two-laser setup was developed to simultaneously trap and monitor a single bacterium in the near-field of a nanobeam microcavity. While the first laser is used to excite the optical field tweezing the bacterium, the second laser probes the cavity resonance spectrum. The bacterium optical interaction with the resonant cavity mode allows to assess the bacterium state in real time when subjected to stress, through the monitoring of the membrane refractive index.

Results: The interaction between the microresonator and the trapped bacterium allows the close inspection of bacterial membrane properties. First, we investigated the distinction between Gram-positive and Gram-negative, on single-cells in a label-free and non-destructive way. Then different levels of thermal stress are directly linked to the variation of the refractive index of the membrane. Compared with culture-based methods, the integrated device and associated methodology developed here show how the measurement of local optical properties provides crucial insights for bacterial membrane monitoring.

PM035 Application of a novel acidophilic zero-valent sulfur-respiring Firmicute in a novel hybrid low pH sulfidogenic bioreactor

Roseanne Barata Holanda[1], D Barrie Johnson[1]

[1]*Bangor University, School of Natural Sciences, Bangor, United Kingdom*

Background: Biosulfidogenesis has been extensively studied for applications in metal recovery processes and bioremediation of metal-rich waste waters. These systems often use species of neutrophilic sulfidogens that need to be protected against direct contact with elevated concentrations of hydronium ions (H_3O^+) present in acidic metal-rich effluents. Currently, there are only few reports describing species of sulfidogenic bacteria that grow at pH below 3.

Objectives: Study the characteristics of a novel acidophilic zero-valent sulfur (ZVS)-reducing *Firmicute*, and its application in a novel "hybrid" low pH sulfidogenic bioreactor (HSB) designed to remediate and recover metals from metal-rich waste waters.

Methods: Sediments from an abandoned copper mine were enriched for acidophilic ZVS-reducers. From these, a novel obligately anaerobic bacterium, strain I2511, was isolated, its whole-genome sequenced and its physiology characterised. The HSB was operated using both ZVS and sulfate as electron acceptors, glycerol as electron donor, and was populated with isolate I2511 and other acidophilic bacteria.

Results: Isolate I2511 grew via ZVS respiration over a wide pH range (1.8 - 5.0), catalysed dissimilatory reduction of Fe^{3+} but was not able to reduce sulfate, thiosulfate or tetrathionate. It displayed low genome relatedness in terms of conserved proteins to currently validated species of acidophilic *Firmicutes*, and was considered to represent a novel candidate genus. The HSB generated H_2S between pH 2 and 7, and when operated at pH 2.0 - 2.5, isolate I2511 was the dominant member (up to ~55% relative abundance) of the microbial population. Results confirmed the potential of I2511 for biosulfidogenic applications.

PM036 Shewanella sp. O23S: versatile aerobic and anaerobic selenium reducer of industrial relevance

Lucian Staicu[1], Maria Dabrowska[1], Lukasz Drewniak[1], Anna Ruszczynska[2], Ewa Bulska[2], Lukasz Dziewit[1]

[1]*University of Warsaw, Faculty of Biology, Warsaw, Poland*
[2]*University of Warsaw, Faculty of Chemistry, Warsaw, Poland*

Background: Bacteria are involved in the biogeochemical cycle of selenium (Se) being a key factor in the cycling of the element in Nature. Specialized bacteria show high selectivity towards selenium present in complex solutions (industrial effluents) because they employ enzyme systems with high affinity for this element. One such strain is *Shewanella* sp. O23S, isolated from a metalliferous environment, an ancient gold mine in Złoty Stok (Poland).

Objectives: i) characterize Se transformations under aerobic and anaerobic conditions in *Shewanella* sp. O23S, ii) test it to clean up and harvest resources from a real selenium-laden industrial effluent.

Methods: IC-ICP-MS for Se speciation, DLS for size and surface charge of Se(0), HPLC for assessing the respiratory process.

Results: Aerobically, the strain withstands excessive concentratios of toxic SeO_3^{2-} (MIC: 120 mM) and SeO_4^{2-} (MIC: 600 mM), accompagned by the formation of red elemental Se, Se(0). Under anaerobic conditions, it sustains growth by lactate oxidation to acetate, concomitently with the respiratory reduction of Se oxyanions to red Se(0). *Shewanella* sp. O23S reduces SeO_4^{2-} to Se(0) directly, bypassing intermediary SeO_3^{2-}, this constituting an unprecedented result. Next, the strain was incubated in a real high-sulfate polymetallic industrial effluent to test its bioremediation potential. The strain preferentally respires Se over sulfate (10,000x in excess of Se), showing great promise for bioremediation. Additionally, resource recovery of a valueble material, Se(0), can be achieved from these effluents. *Shewanella* sp. O23S is the most versatile Se reducer reported so far, having also industrial relevance for decontaminating toxic Se-laden industrial effluents.

PM037 Analysis of the Escherichia coli hydrogenases as anodic enzymes in bioelectrochemical system

Anna Poladyan[1], Tatiana Semashko[2], Liudmila Zhukouskaya[2], Volha Dziameshka[2], Armen Trchounian[1]

[1]Yerevan State University, Biochemistry, Microbiology and Biotechnology, Yerevan, Armenia
[2]Institute of Microbiology, National Academy of Sciences, Belarus, Laboratory of enzymes, Minsk, Belarus

Background: Global energy consumption increases every year. Biofuel cells (BFC) generate electricity during oxidation of organic components and can be an alternative source of energy. Microbial cell components (whole cells, enzymes, membranes) can be used as catalysts in BFCs. Of particular interest is the development of BFCs based on [Ni-Fe] hydrogenases (Hyds). Hyds of *Escherichia coli* have a unique ability to H_2 oxidation and formation which allows using them both as anodic and cathodic enzymes.

Objectives: The purpose of this study is to study the effectiveness of using *E. coli* Hyds as an anode enzymes in bioelectrochemical system with different redox mediators.

Methods: *E. coli* K12 were grown under anaerobic conditions. Bacterial cell extracts were obtained by sonication. *E. coli* whole cells and cell extracts immobilized onto sensors using polyvinyl acetate. Ferrocene and its derivatives (ferrocene-carboxy-aldehyde, ferrorocene-carboxylic acid, methyl ferrocene-methanol) were used as mediators.

Results: Depending on the mediator and H_2 concentration, the current strength varied from 1 to 30 μA. Upon using the ferrrocene-carboxylic acid as mediator, the current strength was enhanced. The dependence of the strength of the current produced on the concentration of H_2 was shown. The maximal value of current strength (up to 150 μA) was achieved with a two fold increased H_2 supplementation. Thus, results show *E. coli* K12 whole cells and cell extracts synthesizing Hyds establish the electrochemical activity as anodic catalysators; derivatives of ferrocene can be used as mediators. The best performance in terms of amperage was observed when using ferrrocene-carboxylic acid.

PM038 the role of seed endophytes on hyperaccumulator seeds germination under heavy metal stress

Haiyan Li[1], Shobhika Parmar[1]

[1]*Kunming University of Science and Technology, kunming, China*

Background: Seed endophytes (SE) are benefit to host plants stress tolerance. However, little is known about the SE of hyperaccumulator. *Dysphania ambrosioides* and *Arabis alpina* are hyperaccumulators that grows at heavy metal (HM)-contaminated sites in Yunnan Province, China.

Objectives: To understand the role of SE of hyperaccumulator on seeds germination under HM stress.

Methods: Six SE from two hyperaccumularors were selected and tested for seeds germination enhancement under multi-HM stress (600, 800 and 15 μg/ml Pb, Zn and Cd). Results indicated that the isolate FZT214 from *D. ambrosioides* showed the best seeds germination improvement. Further work indicated that at the series of Pb stress (200, 400, 600, 800 μg/ml), the germination rate of seeds inoculated with FZT214 (E+) were higher than E- (non-inoculation of FZT214), and showed the significant difference at 800μg/ml ($P<0.005$, *t*-test, n=3). Similarly, at the series of Cd stress (1, 5, 15, 30 μg/ml), the germination rate of E+ were significantly higher than E- ($p<0.05$) except at 15μg/ml concentration ($p>0.05$). However, it was surprisingly that at the series of Zn (400, 600, 800, 1000 μg/ml) stress, the germination rate of E+ were not increased when compared with E-.

Results: The isolate FZT214 was found to be able to enhance germination rate of *D. ambrosioides* seeds under Pb, Cd and multi-HM stress. These findings suggest that the SE of hyperaccumularor may play a key role in host plants survival in natural environment where the soil was contaminated with HM. Therefore, they have a potential application in field phytoremediation.

PM039 Photoinactivation of oral biofilms using visible light and water-filtered infrared A (VIS+wIRA) in combination with indocyanine green (ICG)

Ali Al-Ahmad[1], Thomas Burchard[2], Lamprini Karygianni[3], Marie Follo[4], Elmar Hellwig[5]

[1]Faculty of Medicine, Albert-Ludwigs-University, Department of Operative Dentistry, Freiburg, Germany
[2]Faculty of Medicine, Albert-Ludwigs-University Freiburg, Department of Operative Dentistry, Freiburg,
[3]University of Zürich, Clinic of Preventive Dentistry, Periodontology and Cariology, Zürich, Switzerland
[4]Faculty of Medicine, Albert-Ludwigs-University Freiburg, Department of Hematology, Oncology and Stem Cell Transplantation, Freiburg, Germany
[5]Faculty of Medicine, Albert-Ludwigs-University Freiburg, Department of Operative Dentistry and Periodontology, Freiburg, Germany

Background: Due to the increasing antibiotic resistance, alternative antimicrobial treatments such as antimicrobial photodynamic therapy (aPDT) are required. Most publications relating aPDT with ICG used diode lasers and are restricted to examinations of planktonic bacteria.

Objectives: The present study investigated the effect of visible light and water-filtered infrared A radiation (VIS+wIRA) in combination with indocyanine green (ICG) on different bacterial strains and salivary microorganisms in vitro as well as on the initial and mature oral biofilm grown in situ.

Methods: The irradiation of the samples with VIS+wIRA was conducted for five minutes in combination with different concentrations of ICG. Treatment with chlorhexidine (CHX) served as a positive control. The number of colony forming units (CFU) and bacterial vitality (within biofilms) were quantified using live/dead staining and confocal laser scanning microscopy (CLSM).

Results: All tested bacterial strains and salivary bacteria were killed at a level of 3 log10. The CFU of the initial biofilms were reduced up to 4.3 log10 and of the mature oral biofilms at a range of 2.5 log10 whereas the killing effect of CHX was lower (3 log10 and 1.5-2 log10, respectively). The high bactericidal effect of aPDT against the initial and mature oral biofilms was confirmed by live/dead staining. CLSM images showed a high penetration of ICG using aPDT within the mature biofilm.

Taking the healing effects of wIRA on human tissue into consideration, the aPDT using VIS+wIRA and ICG has the potential to treat periodontitis and peri-implantitis as an adjunct therapy.

PM040 Depleted uranium isotopic fractionation by microalgal: evidence of biologically-mediated re-enrichment

Beatriz Baselga Cervera[1;2], Camino García-Balboa[1], Hector M. Díaz-Alejo[1], Paloma Martinez-Alesón[1], Victoria López-Rodas[1], Eduardo Costas[1]

[1]*Complutense University of Madrid, Madrid, Spain*
[2]*University of Minnesota, Ecology, Evolution and Behavoir, St. Paul, United States*

Background: Depleted uranium tails, by-products from nuclear fuel chain, are highly toxic waste with certain potential of energy use. The residual ^{235}U fissile isotope contained in depleted uranium tails has been recovered only marginally. Changes in the relative isotopes abundances of elements are often induced by biological processes. Regarding uranium isotopic fractionation, it has been previously described in reducing bacteria and neuron-like cells.

Objectives: The conceptual basis of this work is to highlight the ability of two microalgal species to fractionate depleted uranium dissolved.

Methods: Two Chlorophyta microalgal strains, a freshwater *Chlamydomonas sp.* (ChlGS) isolated from an acid uranium tailings pond and marine *Tetraselmis mediterranea* (TmmRU) from the Mediterranean Sea, were exposed to media supplemented with depleted uranium. Twelve replicates of each strain were propagated under depleted uranium pollution for 24 days. Uranium uptake performance and $n(^{235}U)/n(^{238}U)$ ratios were analyzed at different intervals along the bioassays. Uranium isotopic ratios were analyzed in a double-focusing sector field ICP-MS (Element 2, Thermo Scientific).

Results: Depleted U was uptaken by both studied strains, inducing U isotopic fractionation. The $^{235}U/^{238}U$ isotopic fractionation magnitudes (ε) obtained in the cellular pellet were; 23.62±12.47‰ for the freshwater strain and 370.39±103.86‰ for the marine microalgae. The two microalgal strains fractionate depleted uranium and show an enrichment in the fissile ^{235}U isotope in the cellular pellet.

PM041 Encapsulation of Probiotic Bacteria with alginate biopolymers for Production of fermented dairy products

Samadhan Dahikar[1]

[1]*Sanjivani Arts, Commerce and Science College, Department of Microbiology, Kopargaon, India*

Background: The encapsulation techniques for protection of bacterial cells have resulted in greatly enhanced viability of these microorganisms in food products as well as in the gastrointestinal tract.

Objectives: The objective of the study was to improve the functional, nutritional, and rheological properties of dairy products, namely yoghurt.

Methods: In the present study fermented type dairy i.e yoghurt were produced adding free and microencapsulated probiotics. For this purpose probiotic L. brevis bacterial cells were encapsulated in sodium alginate by an encapsulator using electrospinning method. In this study, scanning electron microscopy (SEM), XRD, Fourier Transform Infra Red (FTIR) spectroscopy, and Nuclear Magnetic Resonance (NMR) techniques were used to characterised the encapsulated nanofiber.

Results: The research has been by determining the viability and survival rate of the encapsulated bacterium which was treated in two different ways. At this point of the research, the viability of the Encapsulated bacteria (L. brevis) were observed at a great rate (85%) which was as excellent as in the survival and growth rate in the peptic treatment (63%). In contrast, the viability and survival rate in vivo assay of the free bacteria were far lesser than the rate in its counterpart, the encapsulated bacteria. The study concluded that new developments in microencapsulation by the use of electrospinning technology will allow more control and have the potential of increased benefits.

PM042 Heterologous expression of the lantibiotic nisin O cluster from the human gut bacterium Blautia obeum A2-162

Cristina Gherghisan-Filip[1], Diane Hatziioanou[1], Gerhard Saalbach[2], Nikki Horn[1], Udo Wegmann[1], Sylvia H. Duncan[3], Harry Flint[3], Arjan Narbad[1], Melinda J. Mayer[1]

[1]*Quadram Institute Bioscience, Gut Microbes and Health, Norwich, United Kingdom*
[2]*John Innes Centre, Norwich, United Kingdom*
[3]*Rowett Institute, Gut Health Group, Aberdeen, United Kingdom*

Background: The peptide lantibiotic nisin is an effective antimicrobial used in food and veterinary applications. Several variants have been described, and genetic modification has been shown to improve activity and host range. There is interest in identifying similar bacteriocins from gut bacteria, which should have evolved to function in the challenging conditions of the gastrointestinal tract and could provide new antimicrobials to combat gut pathogens.

Objectives: We identified an unusual variant of the nisin A gene cluster – *nso* - in a human gut isolate, *Blautia obeum*. We aimed to use heterologous expression in *Lactococcus lactis* to produce nisin O peptides and analyse their activity.

Methods: The nisin O gene cluster was expressed on a plasmid in *L. lactis* strains. Production of prepeptides was confirmed by western analysis and mass spectrometry, and prepeptides were concentrated by immunoprecipitation. Tryptic peptides were analysed by LC-MS/MS. Antimicrobial activity was tested using overlay assays.

Results: The nisin O gene cluster has four structural peptides, the first three of which are identical, but no protease to cleave the leader of the structural peptide. Expression of *nso* in *L. lactis* initially failed to give antimicrobial activity. However, expression in a strain containing the nisin A regulatory *nisRK* genes gave increased production of the prepeptides upon nisin induction, and incorporation of trypsin into overlay assays allowed the demonstration of antimicrobial activity against gut pathogens *Clostridium perfringens* and *Clostridium difficile*. LC-MS/MS analysis of tryptic peptides demonstrated dehydration and ring formation and the presence of fully modified cleaved products.

PM043 Evaluation of bacterial isolates for agriculture, with beneficial properties on plant growth and crop protection

Ivica Dimkić[1], Nikola Unković[1], Milica Ljaljević Grbić[1], Slaviša Stanković[1], Djordje Fira[1], Vittorio Venturi[2], Giuliano Degrassi[3]

[1]*University of Belgrade Faculty of Biology*
[2]*International Centre for Genetic Engineering and Biotechnology, Bacteriology*
[3]*International Centre for Genetic Engineering and Biotechnology, Industrial Biotechnology Group IBIOBA-ICGEB*

Background: A diverse array of bacteria, including *Pseudomonas* species are the main plant growth promoting (PGP) agents used to promote the growth of several crops. Many of these isolates are recognized for their PGP ability and capacity to increase disease resistance of the plants.

Objectives: This study aimed to isolate and characterize different rhizospheric, epiphytic and endophytic isolates from the soybean, maize, wheat and barley, and to evaluate their PGP traits, biofilm formation and antifungal activity.

Methods: According to the 16S rRNA gene sequencing and from various PGP traits, 48 out of 100 isolates from the leaves, stems, roots, and seeds from different crops were selected. All the strains were additionally identified (*gyrB*, *rpoB*, and *tufGPF*), and 16 selected isolates marked as belonging to Risk Class 1 group, screened for *in vitro* antifungal activity (*Rhizoctonia solani, Sclerotinia sclerotiorum, Monilinia laxa, Alternaria alternata, Alternaria infectoria, Fusarium graminearum, Fusarium verticilioides, Epicoccum nigrum*) and for biofilm formation.

Results: Thorough housekeeping genes sequencing highlighted representative isolates such as *Pseudomonas fluorescens/putida/fulva/stutzeri/rhodesiae* and *Bacillus subtilis*. The phosphate solubilization, siderophore and IAA production were most related to *P. fluorescens/putida* species, while strong lipase and protease activity are recorded for *P. rhodesiae* and *P. fluorescens*. Strong biofilm production was obtained for *P. fulva*, and moderate for *B. subtilis.* Other isolates showed no biofilm production ability. Only *B. subtilis* showed strong antifungal activity against all tested fungi, and two isolates from *P. fluorescens/putida* group. Most of the *Pseudomonas* isolates expressed strong activity against two *Alternaria* species and *Epicoccum nigrum*.

PM044 Quantification of metal-resistance genes of the microbial community of soils from a uranium mine area

Catarina Marques[1], Jérémie Béguet[2], David Bru[2], Fabrice Martin-Laurent[2]

[1]*Universidade de Aveiro, Departamento de Biologia & CESAM (Centre of Environmental and Marine Studies), Aveiro, Portugal*
[2]*UMR Agroécologie, Dijon, France*

Background: Bacteria can thrive in environments presenting unique, extreme and/or stressful conditions, partly due to the diverse resistome they evolved as a result of physiological, metabolic and genetic adaptations. In this context, the microbial communities in soils from abandoned uranium mines may have peculiar genetic profiles that sustain valuable metal-resistance mechanisms. Getting further knowledge on the genetic potential of these microbial communities can be a contribute for developing bioremediation strategies towards the reclamation of highly contaminated uranium mine soils.

Objectives: To quantify metal-resistance genes harboured by the microbial community of soil samples collected in an abandoned Portuguese uranium mine.

Methods: Microbial DNA was extracted (ISO/CD 11063) from soil samples collected in a reference site and in 5 sampling sites located progressively more distant from the mine area. The abundance of the functional genes involved in metal-resistance mechanisms, *copA* (Cu resistance) and *sodA* (overall stress response) was estimated through quantitative PCR (qPCR) (ISO/FDIS 17601), and expressed relatively to the respective number of copies of 16S rRNA gene or Gammaproteobacteria-specific 16S rRNA gene. Soil metal concentrations were determined by inductively coupled plasma.

Results: The profile obtained for both metal-resistance genes, either relatively to universal 16S or Gammaproteobacteria gene abundances was similar, thereby suggesting the dominance of Gammaproteobacteria in soil samples. The gene *sodA* was more abundant than *copA*, irrespectively of the soils. Notwithstanding, the number of copies of *sodA* was surprisingly higher in the soils located outside the mine area. Apparently, the microbial community is already modulated by the contamination profile.

PM045 Metal bioleaching from chip carrier by Acidithiobacillus spp

Chih-Ching Chien[1], Wing-Sze Ho[1]

[1]*Yuan Ze University, Graduate School of Biotechnology and Bioengineering, Taoyuan, Taiwan*

Background: Bioleaching is the extraction of metal from solid minerals into a solution facilitated by the metabolism of certain microorganisms. *Acidithiobacillus* spp. are well known players in bacterial bioleaching. Aside from pyrometallurgical and hydrometallurgical methods, bioleaching provides another effective technology for metal extraction from low-grade ores and mineral concentrates and can be applied to extraction of metals from electronic wastes (e-wastes).

Objectives: In this study, cultivation of *Acidithiobacillus* spp. using different media will be evaluated. The efficiency of metal bioleaching from chip carrier or e-wastes by *Acidithiobacillus* spp. was examined.

Methods: *Acidithiobacillus* spp. (*A. ferridurans*, *A. thiooxidans* and *A. caldus*) were purchased from Bioresource Collection and Research Centre, FIRDI, Hsinchu, Taiwan. Cultivation of *Acidithiobacillus* spp. were conducted in various Thiobacillus media (e.g. 9K, TSM I and MTFM). Bioleaching of metals from chip-carrier (nickel, copper and gold) were carried out in 500 ml flasks containing 200 ml medium and sterilized chip-carrier with and without cells of *Acidithiobacillus* spp.

Results: Preliminary results indicated that relative to controls, bioleaching process could be facilitated in the presence of *Acidithiobacillus*. There was more nickel (ca. 1.2X) and copper (ca. 1.2X-2.2X) dissolution in the presence of microorganisms compared to that without bacteria while insignificant difference in dissolution of gold was observed.

PM046 Electricity generation by Thermincola ferriacetica with carbon monoxide as the only electron donor

Alexandra Popova[1], Ivan Elizarov[1], Maria Prokofeva[1], Elizaveta Bonch-Osmolovskaya[1;2], Sergey Gavrilov[1]

[1]*Winogradsky Institute of Microbiology, Research Center of Biotechnology RAS, Moscow, Russian Federation*
[2]*Faculty of Biology, Lomonosov Moscow State University, Moscow, Russian Federation*

Background: Waste recycling coupled to electricity generation by microorganisms is an actual challenge nowadays. Bioconversion of carbon monoxide emitted from domestic waste incineration and as syngas in microbial fuel cells (MFCs) is one of the promicing technologies. Electricity production linkeded to CO oxidation was described for microbial consortia grown on organic substrates. The purpose of our study was to reveal a microbe coupling CO oxidation and electricity production without addition of organics. We tested this ability in *Thermincola ferriacetica*, known for hydrogenogenic carboxydotrophy, Fe(III) reduction with CO, and electrogenesis with organic substrates.

Objectives: The main objective was to assess the elecrogenic activity of *T. ferriacetica* grown autotrofically with CO as the only electron donor and carbon source.

Methods: Anode compartment of a two-chamber MFC with stainless steel electrodes was inoculated with *T. ferriacetica*. Anode was poised at -100 mV vs SHE. CO served as the sole carbon source and electron donor. MFC was incubated at 60°C. Cell growth was monitored by microscopic counting. CO consumption and H_2 production were monitored by GC.

Results: *T. ferriacetica* culture generated electricity, growing autotrophically on CO. 7.53 mM CO were consumed, produced 6.09 mM CO_2 along with continuous current density generation of 17-36 mA/m^2 within 100 hours. Electrogenesis correlated with CO consumption and the growth rate, clearly indicating metabolic link between CO oxidation and electricity production in a pure culture of *T. ferriacetica*. This is the first report on CO oxidation in a pure culture of an electrogenic bacterium.

Supported by RSF project #17-74-30025.

PM047 Whole-genome sequencing to investigate two concurrent outbreaks of Salmonella Enteritidis in South Africa

Anthony Smith[1], Nomsa Tau[1], Ntsieni Ramalwa[1], Phuti Sekwadi[1], Premi Govender[2], Vusi Mbuthu[2], Krishnee Moodley[3], Chetna Govind[3], SHAMEEMA KHAN[4], Moherndran Archary[5], Theesan Vedan[6], Juno Thomas[1]

[1]*National Institute for Communicable Diseases*
[2]*Department of Health, South Africa*
[3]*Lancet Laboratories, South Africa*
[4]*Ampath Laboratories, South Africa*
[5]*University of KwaZulu-Natal, South Africa*
[6]*National Institute for Communicable Diseases, South Africa*

Background: Source attribution of foodborne disease outbreaks requires epidemiological investigations augmented by molecular analysis of clinical and food isolates to confirm genetic relatedness. In November 2018, two concurrent outbreaks of *Salmonella* Enteritidis gastroenteritis occurring in the same South African district were investigated.

Objectives: To use whole-genome sequencing (WGS) analysis of bacterial isolates to assist the epidemiological investigation of two concurrent outbreaks.

Methods: Outbreak A was associated with customers (n=16) that breakfasted at the same restaurant, while outbreak B was associated with children (n=27) attending a day-care facility. *Salmonella* Enteritidis was isolated from stool samples in both outbreaks (12 customers and three food handlers in outbreak A; four children in outbreak B). In outbreak A, of several restaurant food items tested, *Salmonella* Enteritidis was isolated from three (eggs, hollandaise sauce and rocket). Available isolates from both outbreaks (n=14) were investigated using WGS using Illumina MiSeq technology. Sequencing data were analyzed at the Enterobase web-based platform, including core-genome multi-locus sequence typing (cgMLST) analysis.

Results: Isolates with epidemiological links to the restaurant (n=11) and the day-care facility (n=3), were shown by cgMLST to be probably genetically related, with no more than five allele differences between each other. On food history, eggs and/or hollandaise sauce were the common food items consumed by ill restaurant customers. Although *Salmonella* Enteritidis was reportedly isolated from the eggs and hollandaise sauce, these isolates were not available for WGS. The cgMLST findings suggest the possibility of a common contaminated food source in both outbreaks and has prompted ongoing investigation.

PM048 Magnetotactic bacteria and magnetosomes against cancer

Lucia Gandarias[1;2], david gandia[2;3], lide arana[4], david muñoz[1;2], itziar alkorta[4], alfredo garcia-arribas[2;3], ana garcia-prieto[3;5], maria luisa fernandez-gubieda[2;3], alicia muela[1;3]

[1]Universidad del Pais Vasco (UPV/EHU), Immunology, Microbiology and Parasitology, Leioa, Spain
[2]Universidad del País Vasco (UPV/EHU), Electricity and Electronics, Leioa, Spain
[3]BCMaterials, Leioa, Spain
[4]Universidad del Pais Vasco (UPV/EHU), Biochemistry and Molecular Biology, Leioa, Spain
[5]Universidad del Pais Vasco (UPV/EHU), Applied physics II, Bilbao, Spain

Background: Magnetotactic bacteria (MTB) are a diverse group of microorganisms that have the ability to synthesize membrane-enclosed magnetic nanoparticles called magnetosomes that present fascinating magnetic properties. Therefore, both MTB and, especially magnetosomes, have been proposed for diverse biomedical applications.

Objectives: In this work we study the efficiency of magnetosomes and, in an innovative manner, of the whole MTB as heating agents in magnetic hyperthermia for cancer treatment. For this, we first study the interaction of both bacteria and magnetosomes with human lung carcinoma cells and determine if these agents cause a cytotoxic effect on them. Then, we apply an alternate magnetic field and verify if it causes a reduction on cell viability.

Methods: To verify that magnetosomes and MTB certainly enter cancer cells, we use different microscopic techniques. Once this has been assured, we use different endocytosis inhibitors to identify the route by which cancer cells internalize these agents using flow cytometry. For the study of the cytotoxic effect that magnetosomes and MTB could cause on cancer cells we incubate them together and check cell viability at different times with flow cytometry and proper Live/Dead stains. Finally, we assess the effectivity of magnetic hyperthermia by applying an alternate magnetic field to cells that have previously internalized magnetosomes and MTB, and checking cell viability afterwards.

Results: Human lung carcinoma cells internalize both magnetosomes and magnetotactic bacteria without suffering any decrease in their viability. The magnetic hyperthermia treatment is effective as it reduces significantly the number of cells present in the culture.

PM049 Slow-growing microbes as cell factory for aroma production

Oscar van Mastrigt[1], Tjakko Abee[1], Soren Lillevang[2], Eddy J. Smid[1]

[1]*Wageningen University & Research, Food Microbiology, Wageningen, Netherlands*
[2]*Arla Foods, Arla Innovation Centre, Aarhus N, Denmark*

Background: While most studies focus on fast-growing cultures of microbes, in natural environments nutrients are scarse leading to slow growth of microbes. Also in food fermentation processes, such as cheese ripening, lactic acid bacteria (LAB) encounter long periods of nutrient limitation. Particular LAB survive these periods of extremely slow growth while still contributing to flavour formation in the fermented product.

Objectives: The aim of this study was to explore the physiological adaptions of *Lactococcus lactis* to extreme energy limitation and to apply the slow-growing cells as cell factories for the production of dairy-related aroma compounds.

Methods: Extreme energy limitation was imposed by prolonged cultivation of *L. lactis* in retentostat bioreactors. During 35 days of retentostat cultivation, we quantified biomass production, cell viability, culturability, metabolite production and plasmid content. Aroma production in retentostat cultures was compared with that in batch cultures and in a milli-cheese model system.

Results: During retentostat cultivation, the growth rate decreased from 0.025 h^{-1} to less than 0.001 h^{-1}. The extreme energy limitation caused the formation of viable but non-culturable cells, which coincided with a 7-fold decrease in the maintenance requirement of the cells. Despite the severe energy limitation, plasmids were maintained and plasmid copy numbers were hardly affected. Finally, we demonstrated that aroma formation by LAB at near-zero growth rates resembled aroma formation during cheese ripening. This study demonstrates the potential of this unique cultivation technique as novel aroma production system and to study slow-growing microbes.

PM050 Discovery of a novel antinematode protein produced by a marine epiphytic bacterium; Pseudoalteromonas tunicata

Nor Hawani Salikin[1;2;3], Suhelen Egan[2;4]

[1]*University of New South Wales, School of Biotechnology and Biomolecular Sciences, Sydney, Australia*
[2]*University of New South Wales, Centre for Marine Bio-Innovation, Sydney, Australia*
[3]*University of Science Malaysia, School of Industrial Technology, Penang, Malaysia*
[4]*University of New South Wales, School of Biological, Earth and Environmental Sciences, Sydney, Australia*

Background: Drug resistance has resulted in an urgent need for the development of new therapies to treat diseases caused by parasitic nematodes. Marine epiphytic bacteria represent a promising yet underexplored source for the discovery of novel antinematode drugs.

Objectives: This project aims to genetically characterise a new antinematode metabolite produced by the marine epiphytic bacterium; *Pseudoalteromonas tunicata* and to unravel the killing mechanism against the model nematode; *Caenorhabditis elegans*.

Methods: To identify the gene(s) responsible for the antinematode activity, we employed a functional genomic screening of *P. tunicata* using *C. elegans* as the target. Specific gene(s) responsible for expressing the antinematode activity were identified through transposon mutagenesis, complementation of mutants with the wild type gene(s) and database homology searches. To unravel its nematode-killing mode, recombinant clones and the extracted protein were assayed against *C. elegans* and nematodes physical destructions were microscopically visualized.

Results: We identified the hypothetical protein; Nematode Killing Protein-1 (designated Nkp-1) (NCBI Accession: ZP_01132246) (235aa) was found responsible for the antinematode activity of *P. tunicata*. Exposure to Nkp-1 clone reduced brood size and nematode viability (<50%) within 96 hours. Whilst Nkp-1 did not match to any characterised protein, it contains a galactose binding domain CBM6 that may covalently interact with the glycoconjugate receptor in *C. elegans.* Exposure to the Nkp-1 caused pharynx distortion, extensive vacuole formation and anal destruction in *C. elegans*. This study paves the way for the development of a novel anthelmintic treatments and highlights the benefit of looking to the marine environment for drug discovery.

PM051 Degradative and proteomic analysis of polyurethane biodegradation by alicycliphilus denitrificans bq1

Jacqueline Fuentes Jaime[1], David Schleheck[2], Javier Cruz-Gómez[3], Martín Vargas-Suárez[1], Herminia Loza-Tavera[1]

[1]Facultad de Química, Universidad Nacional Autónoma de México, Bioquímica, México, Mexico
[2]University of Konstanz, Biology, Konstanz, Germany
[3]Facultad de Química, Universidad Nacional Autónoma de México, Ingeniería Química, México, Mexico

Background: Although polyester polyurethane (PS-PU) is a synthetic polymer that can be attacked by bacterial and fungal esterases, it is not known which other proteins and enzymes are involved in the metabolism of this polymer, specifically in the attack to the urethane bonds.

Objectives: To characterize the biodegradative activity and to analyze the proteomic profile of *Alicycliphilus denitrificans* BQ1 growing in the PS-PU coating Impranil®DLN as the only carbon source.

Methods: Growth was measured as total protein by Bradford assay. FTIR spectroscopy and GC-MS analyses were used to detect modifications of chemical groups and to identify degradation products of the polymer. Urethanase activity was quantified spectrophotometrically by the phenol hypochlorite assay using ethyl carbamate as substrate. Extracellular, cytosolic and membrane proteins extracted from cultures grown with Impranil or acetate were analyzed by differential total protemics.

Results: After 28 hours of incubation in Impranil with strain BQ1, the carbonyl and amide signals of the urethane bonds strongly decreased as detected by the FTIR spectra and generated degradation products such as alcohols, dicarboxylic acids and amines detected by GC-MS indicated hydrolysis of ester and urethane bonds. Urethanase activity was higher in the cytosolic fractions. The proteomic analyses identified Impranil-inducible proteins annotated (predicted) as amidase, asparaginase, N-hydroxylase, alpha/beta-fold hydrolase, fatty-acid β-oxidation enzymes, and many tripartite transporter and ABC transporter proteins, which may be related to the bacterial capacity to attack the functional groups of the polymer and to transport and degrade aliphatic chains of the released monomeric components.

PM052 Cultivation of Lipid Assimilating Yeast Using Rotary Shaking Culture with Inversion

Yukino Kobayashi[1], Nana Kawamura[2], Satoshi Takesono[1], Toshiaki Nakajima[3], Toshiya Shigeno[4], Masayuki Onodera[1]

[1]*Niigata Institute of Technology, Kashiwazaki, Japan*
[2]*PRECI co., Ltd., Tokyo, Japan*
[3]*Tsukuba University, Tsukuba, Japan*
[4]*Laboratory of Tsukuba Environmental Microbiology, Tsukuba, Japan*

Background: We have carried out studies on cultural conditions of *Meyerozyma guilliermondii* TY-89 as lipid assimilating yeast with vegetable oil as carbon source. In rotary shaking culture, the cell growth with Erlenmeyer flask with three baffles (baffled flask) was better than that standard Erlenmeyer flask (standard flask) at the same rotating speed due to improvement of carbon source dispersion with baffles. However, baffled flask is more expensive than standard flask. And then washing of baffled flask is more difficult than that of standard flask.

Objectives: The rotary shaking with inversion increases dispersion of vegetable oil by strong turbulence due to rapid flow change during inversion. We have attempted to increase the rate of cell growth with standard flask in rotary shaking culture with inversion.

Methods: The medium contained commercial vegetable oil as carbon source, ammonium sulfate as nitrogen source, and some minerals. One hundred milliliters of the medium in a 500 ml standard flask was cultured at 30℃ on a rotary shaker (120 rpm). Time of between clockwise and counterclockwise, between counterclockwise and clockwise was 3 seconds, respectively. The cell growth was evaluated using dry cell weight.

Results: The cell growth with standard flask in rotary shaking culture with inversion was almost same as that with baffled flask in general rotary shaking culture. The rotary shaker with inversion seems to be suitable for dispersion of water-insoluble carbon sources.

Acknowledgement: This research was supported by the Wind and Fluid Engineering Research Center, Niigata Institute of Technology.

PM053 Simultaneous oral and vaginal administration of probiotics has the greatest therapeutic effect in case of staphylococcal vaginitis

Lidiia Babenko[1], Liudmyla Lazarenko[1], Liubov Sichel[1], Mykola Spivak[1]

[1]Zabolotny Institute of microbiology and virology of National Academy of Sciences of Ukraine, Problems of Interferon and Immunomodulators, Kyiv, Ukraine

Background: Use of probiotics based on *Lactobacillus* and *Bifidobacteria* strains may be an effective strategy for prevention and treatment of bacterial vaginitis and urinary tract infections. The method of probiotics administration has a significant impact on therapeutic effect and determines the mechanism of their effects and the final result of treatment.

Objectives: The objective was to estimate different methods of administration of *Lactobacillus casei* IMV B-7280, *Bifidobacterium animalis* VKL and *B. animalis* VKB probiotic strains on vaginal and fecal microbiota and cytokines production in case of experimental staphylococcal vaginitis.

Methods: Female BALB/c mice were vaginally infected with *S. aureus* 4325-4 strain and then obtained lyophilized probiotic bacteria vaginally and/or orally.

Results: All methods of probiotic bacteria administration resulted in a decrease of coliform bacteria and microscopic fungi amount in the vagina, but *S. aureus* 4325-4 strain elimination occurred most rapidly under the influence of simultaneous oral and vaginal administration of *L. casei* IMV B-7280 and the composition of three strains, only this way of administration prevented the emergence of *S. aureus* 4325-4 in the intestines. The increase in the number of lactobacilli and bifidobacteria in the vagina was observed after all methods of probiotics administration, in the intestine – only after oral or combined administration. The highest immunomodulatory effectiveness of *L. casei* IMV B-7280 and composition of three strains was also observed after simultaneous oral and vaginal administration – higher level of IFN-γ and IL-12 production and decrease of TNF-α production. Similar results were obtained on the experimental model of candidal vaginitis.

PM054 Minor Genomic Evolution of Staphylococcus aureus During Artificial and Natural Colonization of the Human Nose

Manisha Goyal[1], Fabien Javerliat[2], Mattia Palmieri[1], Caroline Mirande[2], Willem J.B. Van Wamel[3], Mehri Tavakol[3], Nelianne Verkaik[3], Alex Van Belkum[1]

[1]bioMérieux, Data Analytics, La Balme Les Grottes, France
[2]bioMérieux, Microbiology R&D, La Balme Les Grottes, France
[3]Erasmus Medical Center, University of Rotterdam, Department of Medical Microbiology and Infectious Diseases, Rotterdam, Netherlands

Background: *Staphylococcus aureus* is a commensal bacterial species that may become invasive. It primarily lives in the human nasal cavity and can persistently colonize the vestibulam-nasi for long periods.

Objectives: Focus of this study was to determine the short and mid-term genomic evolution of natural and artificial nasal populations of *S. aureus* from two cohorts of healthy volunteers.

Methods: Whole genome sequencing was utilized to examine 84 *S. aureus* isolates to define their relatedness using MLST and SNP analysis. Mutation frequencies between strains have been quantified using SNP divergence assessment. Antibiotic susceptibility and the presence of resistance and virulence genes were also determined.

Results: Naturally colonizing strains showed random divergence pattern with an average of 16 SNPs (range 0-44) over a period of weeks whereas when years were considered these numbers turned out to be 15 (range 9-20). Conversely, during short-term artificial colonization the mutation numbers were even lower i.e. 2SNPs (range 0-5). Quantifying these frequencies, which may also be technological rather than biological in origin (due to sequencing errors), is important for the follow up of outbreaks taking place in restricted time periods, and for setting cut-off values for random versus specific mutations. The adherence gene *cna* was found in two strains depicting that virulence can be acquired over short time periods. No change was observed in antibiotic resistance except one shift from susceptibility to intermediate resistance against erythromycin in a single isolate. This epidemiological investigation elucidated limited genomic heterogeneity among same strains originating from similar ecological niche.

PM055 Chitosan extracted from shrimp residues mediated by lactic fermentation by Lactobacillus plantarum has antimicrobial activity agains yeasts from the bioethanol industry

Isabella Tanganini[1], Ligianne Shirahigue[1], Mariana Altenhofen da Silva[1], Kelly R. F. Muruci de Paula[2], Sandra Regina Ceccato-Antonini[1]

[1]UFSCar, DTAiSER, Araras, Brazil
[2]UFSCar, DCNME, Araras, Brazil

Background: Chitosan is widely used in agriculture and in food, medical and pharmaceutical industries. Despite its potential as antimicrobial, chitosan has not been used in the bioethanol industry yet.

Objectives: To obtain and characterize natural chitosan from shrimp residues obtained by a bioconversion process using *Lactobacillus plantarum* (Lp) and physico-chemical deacetylation, and its potential as antimicrobial against yeasts from the bioethanol process: an industrial strain of *Saccharomyces cerevisiae* (Sc) and a strain of the contaminant yeast *Dekkera bruxellensis* (Db).

Methods: The lactic fermentation was optimized for residue, inoculum and glucose concentrations in MRS medium for higher acid production. Chitin was deacetylated by the autoclave method with NaOH. Chitin and chitosan were characterized by FTIR, XRD, TG-DTA and DSC. Chitosan was evaluated in conditions of growth and fermentation with the yeasts above.

Results: In optimized conditions of Lp fermentation, the demineralization and deproteinization were 77% and 43%, respectively. FTIR analysis confirmed the presence of chitin and chitosan in the samples with 80% deacetylation degree for chitosan. X-ray diffractograms showed the crystallinity profile of the samples (chitin was more crystalline). Higher thermal stability of natural chitosan than commercial chitosan was observed. Chitosan reduced μ_{max} of both yeasts and caused an increase in the lag phase at the concentration of 500 mg/L, but not for Sc. Chitosan added to the fermentation reduced Db number in approximately 1 log cycle after 12 hours, with minor effect in *S. cerevisiae* population. The fermentative efficiency was 22% higher when compared to the fermentation without chitosan.

PM056 Optimization of composting using experimental design towards improving agronomic value and reduction of antibiotic-resistant microorganisms in compost

Flora Ezugworie[1;1], Chukwudi Onwosi[1]

[1]*University of Nigeria Nsukka, Department of Microbiology, Enugu, Nigeria*

Background: Poor waste management options have resulted in serious environmental pollution and disease spread. Composting is a reliable method of waste stabilization because it is ecofriendly and cost-effective. The nutrient-rich end product could be used as biofertilizer. The proliferation of antibiotic-resistant microorganisms in wastes (especially livestock manure) could be effectively reduced using composting.

Objectives: The objectives are to enhance the agronomic value of matured compost by optimizing the proportions of biomass in the compost mix and to reduce the proliferation of antibiotic-resistant microorganisms in organic wastes.

Methods: Poultry litter was co-composted with vegetable waste and corn stalk. The compost mix was optimized with mixture experimental design. Compost maturity was evaluated using parameters such as C: N, electrical conductivity and organic matter. Organic matter degradation was modeled using nth order algorithm. The temperature of the pile was elevated to assess pathogen reduction during composting. MacConkey agar spiked with antibiotics was used to enumerate antibiotic-resistant enterobacteriaceae.

Results: According to the mixture optimizer, the best proportion of compost mix for total organic matter was at 47% poultry litter and 53% corn stalk with the maximum value of 52.74%. The best compost mix for NH_4:NO_3 was at 50% vegetable waste and 50% corn stalk with a minimum value of 1.44. The degradation of organic waste followed the first order kinetics in all the treatments. About 80% reduction was recorded on antibiotics-resistant enterobacteriaceae after composting. Co-composting enhanced the agronomic value of compost thereby making it a good biofertilizer.

PM057 Operation of an aerobic granular system based on pure culture of bacteria for the treatment of nitrate polluted groundwater

Barbara Muñoz Palazon[1,2], Alejandro Rodriguez Sanchez[3], Miguel Hurtado Martinez[2], Alejandro Gonzalez Martinez[1], Jesus Gonzalez Lopez[1]

[1]*Faculty of Pharmacy, Microbiology, Granada, Spain*
[2]*Institute of Water Research, Microbiology, Granada, Spain*
[3]*University of Granada, Departament of Civil Engineering, Granada, Spain*

Background: The increasing use of fertilizers compounds by intensive agriculture has caused the pollution of groundwater bodies by nitrates. The removal of nitrogen compounds is one of the main problems for the water treatments.

Objectives: The aim of this research was design and characterized a new technology based on aerobic granular of pure culture of denitrifying bacteria for the treatment of groundwater polluted with nitrates.

Methods: Four pure cultures of heterotrophic denitrifying bacteria were used as inoculum for start-up of aerobic granular systems (AGS). The AGS were performance in a cylindrical sequential batch reactor. The hydraulic retention time was 10 h and the volume exchange ratio was 80% for each cycle. The temperature was controlled at 25°C. For the synthetic groundwater, two carbon sources were used. The COD, BOD5 and nitrogen oxides were monitored followed the protocol of APHA. The total suspended solids, granular size and settling velocity of the biomass were measured.

Results: The granulation was achieved successful with all denitrifying strains, except Pseudomonas denitrificans. The bioreactors under decreasing concentration of sodium acetate and methanol were operated, and the quality of effluent until 0.4 g L-1 and 0.3 g L-1 , respectively, reached the optimum concentration of nitrate for by european framework for drinking water. The granular biomass was aggregates compact and stable for a long-term operational stability, with a mean size ranging to 8.0-26.0 mm during steady-stable period. The results suggest the suitability of technology at full-scale for the implementation in areas with problems derived from agriculture.

PM058 Identification of FDA-approved drugs targeting the Pseudomonas aeruginosa quorum sensing effector protein PqsE

Valerio Baldelli[1], Francesca D'Angelo[1], Viola Pavoncello[1], Paolo Visca[1], Giordano Rampioni[1], Livia Leoni[1]

[1]*University Roma Tre, Science, Rome, Italy*

Background: In the ESKAPE pathogen *Pseudomonas aeruginosa* the quorum sensing effector protein PqsE is required for full virulence expression and biofilm formation. Therefore, PqsE is considered a good target for the development of anti-virulence drugs able to attenuate virulence and to increase antibiotic susceptibility, especially in biofilm-related infections. Previous studies by our group showed that the drug repurposing approach can be successfully applied to anti-virulence drugs development, reducing time and costs associated with conventional drug discovery processes.

Objectives: The aim of this study was to identify new anti-virulence drugs targeting PqsE by screening a library of 1,600 FDA-approved compounds.

Methods: The screening campaign was performed by using a purpose-built *P*. *aeruginosa* biosensor strain (named Rep-PqsE) in which light emission increases in the presence of a PqsE inhibitor. The effect of selected hits on the expression of PqsE-dependent phenotypes (pyocyanin production, swarming motility, biofilm formation) was validated in the wild type *P*. *aeruginosa* strain PAO1.

Results: Twenty-four hits increasing Rep-PqsE light emission and having no effect on the biosensor growth were found in the primary screening. Subsequent analyses led to the selection of two compounds (PqsE-I2 and PqsE-I3) strongly decreasing the expression of PqsE-controlled virulence phenotypes in PAO1, without affecting growth. Experiments are in progress in order to determine the possible antibiotic adjuvant effect of these anti-virulence drugs in planktonic and biofilm growing cells.

PM059 Prevention and Treatment of Bacterial Skin Infection using Film-forming Agents

Eugenia Sanchez Garcia[1], Kaho Ho[1], Begona Delgado-Charro[1], Albert Bolhuis[1]

[1]*University of Bath, Department of Pharmacy and Pharmacology, Bath, United Kingdom*

Background: Staphylococci are amongst the most abundant skin-colonising bacteria, but also a common causes of nosocomial and community-associated skin infections. One of these, *Staphylococcus aureus* is a commensal bacterium that colonises about 20-30% of the human population. It is also an opportunistic pathogen that can cause severe skin and soft-tissue infections. Over the past decades, the incidence of antibiotic resistant *S. aureus* (e.g. Methicillin-resistant *Staphylococcus aureus* (MRSA)) has risen significantly. One significant problem is, for instance, chronic wound infections in e.g. diabetic patients, leading to increased mortality and associated hospital costs. Here we are testing the development of film-forming agents that act as a physical barrier to prevent bacterial skin infections, which could reduce the cost of treatment and risk of infection.

Objectives: To investigate the antibacterial activities of film-forming agents to prevent and treat attachment and biofilm formation of staphylococci to skin.

Methods: Antibacterial activities of cationic polymers used in pharmaceutical and cosmetic products were evaluated against *S. aureus* (including MRSA). We tested the effect of the polymers on biofilm formation by determining viable count, biomass (crystal violet-based assay), and a resazurin-based cell viability assay.

Results: All the cationic polymers showed noticeable inhibition on the *S. aureus* strains tested. Using a colony biofilm assay on a polycarbonate membrane, the polymers were shown to prevent both attachment and biofilm formation. This indicates that these cationic polymers have considerable antibacterial and antibiofilm activity against *S. aureus*. Further investigations are needed to develop a clinical application in the prevention of biofilm formation.

PM060 Biodegradation of diclofenac by rhodococci: metabolism and cell adaptation

Elena Tyumina[1], Andrea Cartagena Gómez[1], Irina Ivshina[1;2]

[1]Perm State University, Microbiology and Immunology Department, Perm, Russian Federation
[2]Institute of Ecology and Genetics of Microorganisms, Ural Branch of the Russian Academy of Sciences, Laboratory of Alkanotrophic Microorganisms, Perm, Russian Federation

Background: Recently, the ways of pharmaceuticals and their metabolites into the environment as well as changes occurring to these highly persistent and chemically diverse compounds with pronounced biological activities in the open ecosystems are being intensively studied. Actinobacteria of the genus *Rhodococcus* are effective biodegraders of these emerging contaminants.

Objectives: To investigate (1) the kinetics of bacterial degradation of diclofenac (DCF), a polycyclic non-steroidal anti-inflammatory drug, frequently detected in the environment; (2) structural and functional features of rhodococci exposed to DCF; (3) metabolic pathways of DCF biodegradation; and (4) the biological activities of DCF biodegradation products.

Methods: The mechanisms of rhodococcal adaptation to effects of DCF were investigated using an advanced microscopy system of an atomic-force microscope coupled with a confocal laser scanning microscope. DCF and its metabolites were detected by chromatographic and spectral methods; the metabolite bioactivity spectra were predicted using PASS Online.

Results: The comparative bioinformatic analysis of functional genes in the sequenced genomes of *R. erythropolis* IEGM 267 and *R. ruber* IEGM 231 was carried out. The biodegradation kinetics was studied, and a mathematical model of DCF biodegradation was developed. Morphofunctional changes of rhodococci exposed to DCF were revealed. The pathways of bacterial DCF metabolisation were characterized; for the first time, the data confirming the breakdown of the C–N bond and aromatic ring cleavage in the DCF structure were obtained. The anti-inflammatory, antiseborrheic, and antiphobic activities of selected metabolites were detected.

The research was supported by the RFBR grant (17-44-590567) and the RSF grant (18-14-00140).

PM061 Homoacetogenic bacteria differ in their H2 utilization thermodynamics and kinetics: implications for applications

Jo Philips[1]

[1]*Aarhus University, Department of Engineering, Aarhus, Denmark*

Background: Homoacetogenic bacteria are capable of converting CO_2 with H_2 into acetate and ethanol. These microorganisms are therefore of interest for the development of CO_2 converting technologies. The required H_2 can be fed to the bacteria as H_2 gas (i.e. gas fermentation) or by a H_2 generating electrode (i.e. microbial electrosynthesis). Very limited information is available on the thermodynamics and kinetics of H_2 utilization by homoacetogenic bacteria, even though those properties could strongly affect which strains are suitable for which application.

Objectives: The goal of this work was to compare the H_2 utilization thermodynamics and kinetics of different homoacetogenic strains.

Methods: Experimental and theoretical data from literature was collected and analyzed.

Results: Based on recent advances in the understanding of the energy conservation mechanism of homoacetogens, it is expected that the hydrogen threshold (i.e. the lowest hydrogen partial pressure at which homoacetogenesis can proceed) can vary with orders of magnitude between different homoacetogenic strains. In addition, experimental evidence for strong differences in the H_2 utilization kinetics of homoacetogens was found. It can be hypothesized that homoacetogenic strains with a high hydrogen threshold and a high growth rate (e.g. *Clostridium* species) are most suitable for application in gas fermentation. In contrast, homoacetogenic strains with a low hydrogen threshold and strong affinity for low hydrogen concentrations (i.e. *Acetobacterium* species) are likely more suited to consume the low hydrogen levels produced by electrodes during microbial electrosynthesis. Similar differences in H_2 utilization thermodynamics and kinetics could also exist for other groups of anaerobic microorganisms.

PM062 Scattered Light Integrating Collector (SLIC); a revolution in Point-of-Care AST

Robert Hammond[1]

[1]*University of St Andrews, Medicine, United Kingdom*

Background: The burden of Anti-Microbial Resistance (AMR) is a growing problem globally. In the O'Neill report (May 2016) it was stated that one of the key milestones in stopping AMR as a global problem was effective rapid antibiotic susceptibility tests (AST). Here, we present a device that determines susceptibility rapidly and could help turn the tide of AMR. SLIC (Scattered Light Integrating Collector) is a sensitive device for the detection of both bacterial and fungal infections based on the scattering of laser light.

Objectives: To establish a rapid AST test for pure bacterial and fungal culture from plates, from urine in a point-of-care fashion, from positive blood cultures and from primary whole blood.

Methods: Proof of concept studies were carried out initially to establish the lower limit of detection. This was found to be 10-50 cfu/mL. This exquisite sensitivity allowed us to commence work establishing rapid MICs. Starting with an inoculum of 10^5 mL bacteria and using a relevant range of antibiotic concentrations the MIC can be established in less than one microbial doubling period.

Results: The rapid and sensitive detection SLIC affords allows for fast growing organisms such as *E. coli* and *S. aureus* to have their MICs established in less than 30 minutes, for any antibiotic. For slow growing organisms such as *M. bovis* we are able to establish an MIC in <2 hours. The technology can also be used for fastidious and difficult to grow organisms such as *H. influenzae* and *Mycoplasma spp.*

PM063 Investigating the microbial degradation of epoxy resin related material

Lucrezia Pardi-Comensoli[1], Andrea Colpo[1], Zuzanna Palczewska[1], Markus Heeb[2], Andreas J. Brunner[1], Michel Barbezat[1], Giovanni Terrasi[1]

[1]Empa, Swiss Federal Laboratories for Materials Science and Technology, Laboratory for Mechanical Systems Engineering, Dübendorf, Switzerland
[2]Empa, Swiss Federal Laboratories for Materials Science and Technology, Laboratory for Applied Wood Materials, Dübendorf, Switzerland

Background: Epoxy resin is a synthetic thermoset produced by the reaction of a bisphenolic compound with an amine or hydroxide based hardener. This extremely resistant polymer is increasingly used in many engineering applications, such as carbon fiber reinforced materials (CFRP). Due to its high stability against thermal and chemical degradation its recycling is extremely difficult. Therefore an efficient and sustainable methodology for the disposal and/or recycling of this material is still not available. Different strategies are currently under consideration, but these techniques require large amounts of energy, employ aggressive chemicals, or produce an undesirable emission of toxic gases.

Objectives: During evolution microbes have adapted to a range of severe environments developing countless metabolic abilities that enable growth. In this project their potential for the biodegradation of epoxy related materials is being explored. An overview of the preliminary results will be presented here.

Methods: Mass loss quantification, colorimetric, and UV-Visible investigations were carried out on epoxy samples incubated in soil microcosms for 1, 3, and 6 months. On the same samples changes in selected mechanical properties (hardness and flexural strength) and of surface chemistry (Fourier transform infrared spectroscopy and contact angle) were evaluated. Studies of microbial colonization of epoxy samples exposed to water were also performed with environmental scanning electron microscopy (ESEM).

Results: Colorimetric data showed diffenrences on the samples incubated with microbes compared to the sterile controls. In addition, investigation of microbial colonisation on samples incubated in water revealed an aboundant microbial biofilm formation, inducing modifications on the epoxy substrate.

PM064 Selection of wakame assimilative Lactobacilli and in vitro evaluation of their immunomodulatory potentials

Binghui Zhou[1;2], Yuki Masumizu[1;2], Yuhka Nakano[1;2], Yuhki Indoh[1;2], Ryoya Komatsu[1;2], Md. Aminul Islam[1;2;3], Wakako Ikeda-Ohtsubo[1;2;4], Leonardo Albarracin[4;5], Yoshihito Suda[6], Keiichi Suzuki[7], Hisashi Aso[2;4;7], Julio Villena[4;5], Haruki Kitazawa[1;2;4]

[1]Food and Feed Immunology Group, Graduate School of Agricultural Science, Tohoku University, Sendai, Japan
[2]International Education and Research Center for Food and Agricultural Immunology(CFAI), Graduate School of Agricultural Science, Tohoku University, Sendai, Japan
[3]Department of Medicine, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh
[4]JSPS Core-to-Core Program, Japan
[5]Laboratory of Immunobiotechnology, Reference Center for Lactobacilli (CERELA-CONICET), Tucuman, Argentina
[6]School of Food, Agricultural and Environmental Sciences, Miyagi University, Sendai, Japan
[7]Graduate School of Agricultural Science, Tohoku University, Sendai, Japan

Background: Synbiotics, a combination of prebiotics and probiotics, have been considered as potential candidates for antimicrobial substitutes due to the positive effect on gastrointestinal disorders. Wakame, an edible seaweed, has been widely consumed in Japan resulting bulk of waste. The leftover wakame could be used in feed formulation as a prebiotic in combination with immunomodulatory probiotics (immunobiotics).

Objectives: In order to select the wakame assimilating immunobiotics as potential symbiotic candidates, we isolated Lactobacilli from the porcine intestine using a component adjusted wakame broth and agar medium, and screened them in vitro for their immunomodulatory properties including mucin-adhesion and anti-inflammatory ability.

Methods: Isolated Lactobacilli were identified by 16S rRNA sequencing. Biodiversity of microflora was analyzed by T-RFLP method. Mucin-adhesion ability was evaluated by Biacore assay. Fluorescence was measured after co-incubation of labeled bacteria and PIE cells. pH, turbidity, viable bacterial count and sugar consumption were measured following incubation of Lactobacillus strains in the enzyme-treated wakame medium. Anti-inflammatory potential was evaluated by measuring IL-6 expressions in the lactobacilli pre-stimulated PIE cells after ETEC challenge.

Results: A total of 136 Lactobacilli strains were isolated, all of which could assimilate enzyme-treated wakame. Eight strains were selected by the results of anti-inflammatory ability. Three strains showed a higher adhesion ability to sPIM than to sHCM. Only one strain strongly adhered to PIE cells. One strain showed anti-inflammatory ability. Some sugars were consumed by Lactobacilli, indicating that the Lactobacillus strains could utilize the carbon source from Wakame, which often considered as prebiotics.

PM065 Improving the Recovery and Detection of Blood Stream Pathogens from Blood Culture

Kerry Falconer[1], Stephen Gillespie[1], Robert Hammond[1]

[1]*University of St Andrews*

Background: The chance of surviving a bloodstream infection (BSI) rapidly declines every hour appropriate treatment is delayed. Current detection systems are failing to detect BSI quick enough. Most patients wait up to 48hrs before a diagnosis is confirmed. Scattered light integrating collector (SLIC) enables high resolution monitoring of bacterial growth in real-time.

Objectives: Nine different recovery methods were screened to optimise a rapid bacterial detection system directly from blood culture.

Methods: Simulated ATCC 25922 *E. coli* blood culture was used as a model to evaluate 9 different bacterial recovery methods. Each method was assessed on recovery yield, cost, time, risk of contamination and impact on bacterial generation time. The highest scoring recovery method was further evaluated using simulated blood cultures spiked with 7 of the most frequent blood culture pathogens. The recovery yield was calculated based on CFU count before and after each recovery method. Bacterial generation time of recovered bacteria was measured using SLIC.

Results: Screening of the 9 methods highlighted that a short centrifugation using serum separator tubes gave the greatest benefit. Consistent bacterial recovery was possible for all 7 BSI pathogens (-0.67 $\pm$ 0.14). Bacterial generation time was prolonged in blood culture with the exception of *S. aureus* (> 0.62 $\pm$ 0.26). Time to detection on SLIC for recovered BSI pathogens ranged from 5 to 70 minutes.

Identified is a recovery method that enables a high recovery yield and facilitates a rapid detection system for positive blood culture that could potentially save critical time in BSI.

PM066 A new efficient treatment of microcystins for industrial water supplies

Paloma Martinez-Alesón[1], Hector M. Díaz-Alejo[1], Victoria López-Rodas[1], Eduardo Costas[1], Camino García-Balboa[1], Beatriz Baselga Cervera[1]

[1]*UCM Faculty of Veterinary Medicine, Animal Science, Madrid, Spain*

Background: Freshwater has many industrial uses and, usually, water conditioning is needed. Harmful Algal blooms (HABs) represent one of the main causes of water pollution. In freshwater resources, cyanobacterial blooms and concomitant possible toxicity constitute an economic, environmental and health problem. Microcystins (MCs) are powerful and common toxins produced by cyanobacteria and, due to its stability, are difficult to eliminate. Therefore, appropriate industrial water management regarding MCs pollution should be carried out.

Objectives: Our dare was to develop an inexpensive but efficient treatment for industrial water resources to remove MCs present in the water.

Methods: We have isolated several bacterial strains from *Microcystis sp.* HABS and evaluted MCs-LR degradation capacity. Strains were characterized and identify by 16S rRNA PCR. Batch biodegration experiments were carried out with five of the strains exposed to 200 µg L^{-1} MC-LR up to a week. We have also tested in the laboratory four different phyllosilicates (Bentonite, Zeolite, Stevensite, and Sepiolite) and compare them to active carbon for MC-LR retention.

Results: We observed diferences in the biodegradation performace of the strains, but the five strains were able to remove up to 200 µg L^{-1} MC-LR in less than a week. The four phyllosilicates tested were able to retain more Microcystin-LR than the silica sand, being Sepiolite the most efficient alternative to active carbon. The selected material and strain were used to treat two artifical freshwater ponds of a thermosolar plant.

PM067 Mode of action of Bacillus spp. in the control of green mould of Agaricus bisporus

Olja Stanojević[1], Tanja Berić[1], Andrea Milić[1], Ivana Potočnik[2], Slaviša Stanković[1], Svetlana Milijašević-Marčić[2]

[1]Faculty of Biology (University of Belgrade), Chair of Microbiology, Belgrade, Serbia
[2]Institute of Pesticides and Environmental Protection, Laboratory of Applied Phytopathology, Belgrade, Serbia

Background: *Bacillus* species are widely studied as biocontrol agents given their competitive behaviour, ability to synthesise various antimicrobials and form resistant endospores. They represent an eco-friendly manner of crop disease control, including green mould of white button mushroom caused by several *Trichoderma* species.

Objectives: The objectives of the study involved testing of the antifungal activity of *Bacillus* spp. cell-free supernatants against two *Trichoderma* spp. *in vitro* and detecting the lipopeptide biosynthetic genes in tested strains, with the aim of preliminary determination of the mode of action.

Methods: *Bacillus* spp. supernatant activity against *Trichoderma aggressivum* f. *europaeum* T77 and *Trichoderma harzianum* T54 was tested by well-diffusion assay, in four replicates. Detection of four lipopeptide biosynthetic genes (*sfp*, *fenD, bamC*, part of the iturin operon) in *Bacillus* spp. strains was performed by PCR amplification.

Results: Twenty-three *Bacillus* spp. supernatants inhibited the growth of *T. harzianum* T54 with inhibition zone diameters ranging from 16.1 to 25.9 mm while only seven supernatants induced inhibition zones of *T. aggressivum* f. *europaeum* T77 that ranged from 14.3 to 29.3 mm in diameter. Fifteen *Bacillus* spp. strains were positive for the presence of *sfp*, ten for *fenD*, nine for *bamC* gene and six for the partial iturin operon. Nine strains did not show any antifungal activity towards the tested pathogens, although three of them were positive for at least one lipopetide gene. The results suggested antibiosis as a possible mode of action for 24 out of 33 examined *Bacillus* spp. strains.

PM068 Efficiency and mechanisms of Aspergillus flavus inactivation by atmospheric cold plasma and plasma-activated water

Agata Los[1], Dana Ziuzina[1], Daniela Boehm[1], Patrick J. Cullen[1;2], Paula Bourke[1]

[1]Dublin Institute of Technology, Plasma Research Group, School of Food Science and Environmental Health, Dublin, Ireland
[2]University of Sydney, School of Chemical and Biomolecular Engineering, Australia

Background: Cold plasma treatment is a promising intervention in food processing to boost product safety and extend the shelf-life. The activated chemical species of cold plasma can act rapidly against microorganisms at ambient temperatures without leaving chemical residues.

Objectives: Cold plasma treatment is a promising intervention in food processing to boost product safety and extend the shelf-life. The activated chemical species of cold plasma can act rapidly against microorganisms at ambient temperatures without leaving chemical residues.

Methods: Fungal aqueous spore suspensions of *A. flavus* were exposed to direct gas plasma as well as plasma activated water (PAW). Subsequently, numbers of surviving spores were estimated and concentrations of extracellular DNA were measured. The effects of direct plasma and PAW on DNA damage, apoptotic and necrotic death levels and lipid peroxidation were also examined. Additionally, chemical analysis of spore suspensions and PAW was carried out.

Results: Both direct plasma treatment up to 5 min and PAW with contact time up to 24 h resulted in a significant decrease of *A. flavus* spores viability. The chemical characterisation of PAW and aqueous spore suspensions pre- and post-treatment indicated that changes in concentrations of secondary reactive species such as hydrogen peroxide, nitrates, and nitrites, were an important factor of antimicrobial activity. Pronounced effects of plasma on spores surface elements and internal structures were observed, providing new insights into the mechanisms resulting in inactivation of fungal spores by cold plasma.

PM069 Comparison of Phytochemicals, Antioxidant and Antimicrobial Activity of Peels and Juices of Citrus fruit species

Christiana Jesumirhewe[1], Omeke Nnenna Maureen[1], Ogunlowo Oladejo Peter[2]

[1]*Igbinedion University Okada Edo state Nigeria, Pharmaceutical microbiology, Okada, Nigeria*
[2]*Greenlife Laboratories Osogbo, Osun state, Nigeria*

Background: Antimicrobial resistance and the decreasing efficiency of antimicrobial drugs have resulted in the search for antimicrobial agents as an important strategy for the establishment of alternative therapies in handling difficult infections.

Objectives: This study was carried out to compare the phytochemical components, antioxidant properties and investigate the antimicrobial activities of *Citrus* fruit juice and peels (*Citrus aurantifolia, Citrus sinensis* and *Citrus limon*) against *Escherichia coli* isolated from patients with gastroenteritis in Edo state, Nigeria.

Methods: Concentrated juice samples and ethanolic extracts of the peels were obtained. The antimicrobial activities of the resulting crude extracts were screened using agar well diffusion assay methods. Gentamicin was used as a standard in the antimicrobial activity studies. The free radical-scavenging activity of the samples was determined using the stable 2, 2-diphenyl-2-picrylhydrazil (DDPH). The Ferric reducing antioxidant power (FRAP) assay and the β-carotene-linoleate bleaching assay were used to determine the total antioxidant activity in the extracts. The qualitative presence of terpenoids, flavonoids, saponins, alkaloids, tannins and cardiac glycosides were also analysed.

Results: The extract of both the juice and the peels of *Citrus aurantifolia* had the highest antimicrobial activity compared to *Citrus limon* and *Citrus sinensis.* Gentamicin also had better inhibitory effects on the isolates used. The crude extracts of the juice and peels of the fruits exhibited significant antioxidant activity and free radical scavenging effects. The phytochemical tests revealed the presence of various active medicinal constituents analysed. Our study showed good promising evidence for the antimicrobial effects of *Citrus aurantifolia* juice and peels.

PM070 Direct imaging of sub-cellular fluctuations provides a rapid label-free viability test and a new way to observe bacterial response to antibiotics

Charlotte R Bermingham[1], Isabel Murillo[2], Alexandre D J Payot[3], Krishna C Balram[4], Maximilian B Kloucek[1], Simon Hanna[1], Niamh M Redmond[5], Helen Baxter[6], Ruth Oulton[7], Matthew B Avison[2], Massimo Antognozzi[1]

[1], School of Physics
[2], School of Cellular and Molecular Medicine
[3], Department of Aerospace Engineering
[4], Department of Electrical and Electronic Engineering
[5], National Institute for Health Research Collaboration for Leadership in Applied Health Research and Care West
[6], Bristol Medical School
[7], School of Physics and Department of Electrical and Electronic Engineering

Background: Determining the viability of bacteria in a sample is an essential microbiological technique used in healthcare, industry and research. Increasingly, attention has been focussing on antimicrobial (antibiotic) susceptibility tests (AST) to allow rapid and appropriate prescribing of antibiotics. Current AST methods are slow as they rely on detecting the growth of microorganisms, but faster AST could be enabled by the recent discovery that living bacteria manifest nano-scale fluctuations. These fluctuations disappear when the bacteria die.

Objectives: To demonstrate a direct method of visualising bacterial fluctuations using a label-free optical method, termed Sub-Cellular Fluctuations Imaging (SCFI). This new imaging technique is based on Total Internal Reflection Microscopy (TIRM).

Methods: Bacteria are bound to a glass surface using antibodies and illuminated using an evanescent field. SCFI quantifies the magnitude and location of nano-scale fluctuations to determine the state of individual bacteria in a few seconds. Pixel intensities in the analysis area are extracted from each frame and normalised. Welches two-sided t-test for independent samples was used to statistically analyse the fluctuation distributions.

Results: SCFI reveals how nano-scale fluctuations exhibited by living cells are located within approx. 100 nanometres from the outer cell membrane. Furthermore, we show that SCFI can measure the viability of bacteria in a sample within minutes, clearly distinguishing not only between live and dead bacteria but also live bacteria in different metabolic states. Importantly, we show that SCFI can rapidly distinguish antibiotic-treated resistant and susceptible bacteria, providing a comprehensive diagnostics tool with application as a rapid AST.

PM071 Production of fuels and chemicals from seaweeds

Ana López-Contreras[1], Jaap van Hal[2], Bert Groenendaal[3], Anne-Belinda Bjerre[4]

[1]*Wageningen Food and Biobased Research, Bioconversion, Wageningen, Netherlands*
[2]*ECN part of TNO, Petten, Netherlands*
[3]*Sioen Industries, Ardooie , Belgium*
[4]*Teknologisk Institut, Taastrup, Denmark*

Background: Seaweeds (or macroalgae) are aquatic plants that have a long history of use in food, feed, fertilizer and as source of chemicals or food ingredients. The annual production has been estimated in approx. 27 million tonnes seaweeds with a value of the products of US$6 billion, with growing markets and production capacity. Because of their capacity to capture atmospheric CO_2, their special chemical composition and the possibility of large scale cultivation in the ocean or in combination with aquaculture systems (Integrated Multitrophic Aquaculture systems, IMTA) for bioremediation, they are potential feedstocks for production of renewable chemicals and fuels.

Objectives: To define biorefinery routes for seaweed species that native of the North Sea. The conversion of sugars in the seaweeds to acetone, butanol and ethanol (ABE), or isopropanol butanol and ethanol (IBE), with uses as fuels or chemicals was studied.

Methods: As an example, in the green seaweed *Ulva lactuca*, the main sugars are L-rhamnose and D-glucose, that can be fermented into acetone or isopropanol, butanol and ethanol (ABE or IBE), while the protein-rich fraction of *U. lactuca* was characterized as animal feed. The fermentation profile on *U. Lactuca* hydrolysates and mixed sugars by *C. beijerinckii* was studied.

Results: *C. beijerinckii* fermented L-rhamnose into ABE or IBE, plus 1,2-propanediol. The product profile, as well as data on transcriptomics suggests that this strain produces bacterial microcompartments (BMCs) when grown on L-rhamnose. In addition, an overview on seaweed biorefinery approaches by the EU Macrofuels project will be given.

PM072 gc-ms analysis and antibacterial activities of extracts of Laurencia intermedia and Gelidium corneum from West African Coasts

Folashade Agbaje-Daniels[1], Adekemi Faboyede[2], Daniella Obahor[2]

[1]Crawford University, Biological Sciences, , Ogun State, Nigeria
[2]Crawford University, Biological Sciences, Ogun State, Nigeria

Background: Antibiotic resistance of bacterial strains is fast returning man to the pre-antibiotic era. This thus calls for increased research into new drug development. Marine macroalgae have been explored for novel drugs in recent times and have shown great promises as sources of novel compounds.

Objectives: To screen the crude extracts of *Gelidium corneum* and *Laurencia intermedia* from West African Coast for antibacterial activities and obtain the chemical compounds composition

Methods: Crude extracts from *Laurencia intermedia* and *Gelidium corneum* were obtained by maceration using dichloromethane and chloroform (2:1). The extract was concentrated en vacuo. Antibacterial susceptibility test was carried by disc diffusion method while minimum inhibitory concentration was obtained by microdilution. The chemical composition of the extracts was obtained by GC-MS analysis

Results: Both extracts exhibited activities against both Gram positive and Gram negative test bacteria. The maximum inhibitory zones observed were 10mm (*Pseudomonas putrefaciens*) and 9mm (*Pseudomonas putrefaciens* and *Eschericia coli*) in *G. corneum* and *L. intermedia* respectively. *S. aureus* was inhibited with zones of 9mm and 6mm in *C. Corneum* and *L. intermedia* respectively. The MIC obtained for *G. Corneum* ranged from 1.2 µg (*S. aureus*) to 39 µg (*Pseudomonas putrefaciens*) while the MIC for *L. intermedia* ranged from 9.7 µg (*E. coli*) to 1250 µg (*P. flourescenes*). GC-MS result showed the presence of several compouds including Pentadecanal, Phthalic acid, Difluorobenzoic acid and Hexadecanoic acid which had earlier been reported for antibacterial activites. Study revealed that these algal extracts could be antibacterials with promises for novel drug.

PM073 Herbal and Fruit Vinegar as Potential Natural Medicinal Products against Bovine Mastitis Caused by Staphylococcus and Streptococcus Infections

Thararat Chitov[1], Anuttree Inyoo[1], Tanakit Thiyakum[1], Hataichanok Pandith[1], Yingmanee Tragoolpua[1], Sakunnee Bovonsombut[1]

[1]*Chiang Mai University, Department of Biology, Chiang Mai, Thailand*

Background: Bovine mastitis is a major problem in dairy farming, causing reduction in the quality, thus poses significant negative economic impacts on dairy farmers and industry. Mastitis is treated mostly via the administration of antibiotics. However, for organic dairy farms, natural alternative remedies are required.

Objectives: The objective of this research work was to investigate antibacterial activity of herbal and fruit vinegars against major bacteria associated with bovine mastitis.

Methods: Twenty naturally fermented herbal and fruit vinegars were tested against representative strains of bovine mastitis-associated bacteria in genera *Staphylococcus* and *Streptococcus*. *Staphylococcus* species included *S. epidermidis, S. aureus, S. capitis* supsp. *urealyticus, S. chromogenes, S. cohnii* subsp. *cohnii, S. cohnii* subsp. *urealyticus, S. haemolyticus, S. hominis* subsp. *novobioseptiae, S. sciuri,* and *S. simulans. Streptococcus* species included *Str. Agalactiae, Str. dysgalactiae,* and *Str. uberis.* The antibacterial activity of the vinegars after nutralisation was also tested.

Results: Among 20 types of vinegars tested, those brewed with coffee, ginger root, tea leaf, long pepper, bamboo grass, lychee, peach, strawberry, plum, avocado, creeping daisy, cassumunar ginger, and star gooseberry had antibacterial activity against a wide range (>90%) of mastitis-associated bacterial species. The inhibitory effect mainly resulted from acid(s). However, some vinegars, especially creeping daisy and ginger, still retained inhibitory activity to some bacterial species after neutralisation. The results from this study showed that these herbal and fruit vinegars can potentially provide natural medicinal alternatives to antibiotics, especially for organic dairy farms. These vinegars will be further investigated for their anti-inflamatory activity and active compounds.

PM074 Co-production of urethanase and urease by bacteria isolated from putrefying Sargassum wastes

Bidyut Mohapatra[1]

[1]*The University of the West Indies (Cave Hill Campus), Department of Biological and Chemical Sciences, Bridgetown, Barbados*

Background: The occurrence of urethane ($NH_2COOC_2H_5$) in alcoholic beverages, especially in wine, sherry, sake, stone fruits brandy and whisky, is a major concern for the global regulatory agencies owing to its carcinogenicity and teratogenicity. The generation of urethane is primarily attributed to the reaction between urea and ethanol during fermentation, distillation and long-term storage. Recently, the US Food and Drug Administration has been recommending various urethane abatement strategies, including enzymatic treatment via acidic urease (EC 3.5.1.5) to scavenge urea (the principal precursor of urethane). However, once urethane is formed, acidic urease treatment is ineffective. Enzymatic cleavage of urethane to carbon dioxide, ethanol and ammonia can be performed directly by urethanase (EC 3.5.1.75).

Objectives: In this study, efforts were directed to identify the urethanase- and urease-co-producing bacterial isolates from decaying seaweed wastes piled off the Barbados coast and to characterize the extracted enzymes from selected isolates.

Methods: The presumptive urethanase- and urease-producing bacteria were isolated via serial dilutions and spread plate techniques with low peptone medium (LPM, pH 6) fortified with 0.2% urethane or urea, respectively. A rapid screening of the presumptive isolates for urethanase and urease activities were performed in LPM using phenol red staining.

Results: The isolates, which co-produced urethanase and urease activities, were identified via the 16S rRNA gene sequencing approach. The enzymatic characteristics of the co-produced urethanase and urease extracts, including tolerance toward higher concentration of ethanol and acidic pH, suggest the potential of microbial biocatalysts for the abatement of carcinogenic urethane in fermented beverages.

PM075 Development and validation of a reference data set for assigning Staphylococcus species based on next generation sequencing of the 16S-23S rRNA region

Maja Kosecka-Strojek[1], Artur J. Sabat[2], Viktoria Akkerboom[2], Karsten Becker[3], Evert van Zanten[4], Guido Wisselink[4], Jacek Miedzobrodzki[1], Mirjam Kooistra-Smid[2;4], Alexander W. Friedrich[2]

[1]*Department of Microbiology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Krakow, Poland*
[2]*University of Groningen, University Medical Center Groningen, Department of Medical Microbiology, The Netherlands*
[3]*Institute of Medical Microbiology, University Hospital Münster, Münster, Germany*
[4]*Certe, Groningen, Department of Medical Microbiology, The Netherlands*

Background: Many members of the *Staphylococcus* genus are clinically relevant opportunistic pathogens that warrant accurate identification for targeted therapy. A previously developed method based on next generation sequencing (NGS) of the 16S-23S rRNA region is a reliable and precise approach for species identification. However, the introduction of this new method is hindered by a lack of 16S-23S rRNA region reference sequences for many bacterial species, including staphylococcal species.

Objectives: The aim of this study was to develop a 16S-23S rRNA sequence reference set for the identification of staphylococcal species based on NGS of the 16S-23S rRNA region.

Methods: Fifty staphylococcal reference strains were subjected to bacterial identification by Sanger-based sequencing of the genes encoding for 16S rRNA, *sodA*, *tuf* and *rpoB* and NGS of the 16S-23S rRNA region. Additionally, 100 staphylococcal strains and 43 clinical samples were identified by routine diagnostic methods and NGS of the 16S-23S rRNA region.

Results: The study allowed development of a reference sequence dataset of the 16S-23S rRNA region for 50 (including one newly proposed species) *Staphylococcus* species. *Staphylococcus* species could be reliably detected in samples composed of both single species and more complex polymicrobial communities. This study will be useful for introduction of a novel diagnostic tool, which is an improvement for reliable species identification particularly in polymicrobial clinical samples.

This project was supported by the National Science Centre (NCN, Poland) on the basis of the decision no. UMO-2016/21/N/NZ6/00981 and in part by the European Regional Development Fund within the EurHealth-1Health project EU/INTERREG VA-681377.

PM076 A novel protocol for the synthesis of monodisperse sodium alginate beads in a glass microfluidic device for single-cell applications

Lucy Witherall[1], Ula Lapinska[2], Stefano Pagliara[2], Ben Temperton[1]

[1]*University of Exeter, United Kingdom*
[2]*University of Exeter, Living Systems Institute, Exeter, United Kingdom*

Background: Encapsulation of single bacteria in droplets enables high-throughput application of various molecular techniques at single-cell resolution, and is a major leap forward in understanding phenotypic variance in microbial cultures. Alginate has previously been used in polydimethylsiloxane (PDMS) microfluidic devices to produce droplets that are able to encapsulate cells and other biomolecules. However, the negatives of PDMS (single-use, auto-fluorescence and restricted chemical compatibility) outweigh the benefits. Developing a protocol for alginate droplet production in glass microfluidic devices, which can be reused, have low-autofluorescence and improved optical characteristics, is therefore of interest.

Objectives: This study aimed to optimise size and uniformity of alginate droplets, using glass microfluidic 'lab-on-a-chip' technology. This study had the wider intention of advancing the practical use of alginate beads in numerous research areas, including as delivery systems for bacteriophage, or for use in epicPCR, to associate functional genes and viral predation with community members at the single-cell level.

Methods: A glass microfluidic device, with a channel depth of 100 µm and fluorophillic inner coating was used. The device has a simple X-junction, with a water-in-oil emulsion to form droplets. 1 % (w/w) aqueous sodium alginate was used inside a carrier oil phase and formed droplets were put directly into 2 % (w/w) calcium chloride solution.

Results: Presented here is an improved yet simple protocol for producing monodisperse water-in-oil sodium alginate droplets. This method improves the efficiency of the production of alginate beads, for use across a variety of fields, including microbiological research, bacteriophage therapy and single-cell genomics.

PM077 Rapid and ultrasensitive bacteria and bacterial spore detection by non-lytic ATP elimination and bioluminescent detection

Subhanjan Mondal[1], Kevin Hsiao[1], Said Goueli[1]

[1]*Promega Corporation, R&D, Fitchburg, United States*

Background: Detection of intracellular ATP using firefly luciferase has been utilized in rapid microbiological methods. However, extracellular ATP reduces the sensitivity and bacterial spores are resistant to typical extractants.

Objectives: We hypothesized the use of a tunable ATPase and a spore germinator solution for ultrasensitive detection bacterial and bacterial spore.

Methods: We purified an ATPase that can be inhibited without affecting the firefly luciferase. The enzyme acted on both ATP and ADP to generate AMP with a specific activity of 10 μmoles/min/mg for hydrolysis of ATP. Since spore-coat of a bacterial endospore is highly resistant to chemicals, a spore germination solution consisting of glucose, alanine in PBS allowing shedding of the spore-coat and action by a lytic agent. The lytic agent included ingredients for concurrent inhibition of the ATPase and extraction of cellular ATP. The ultrasensitive ATP Detection Reagent relies on a thermostable luciferase developed through directed evolution.

Results: We found that even in the presence of 1uM extracellular ATP, detection more than 100 CFU/ml was achieved. We then used the system to quantify the average ATP content from different bacteria; *Bacillus subtilis* spores, *Pseudomonas aeruginosa* and *Staphylococcus aureus* to be 1.5, 1.7 and 2.6 attomole/CFU respectively. We then used an upfront filtration and performed ATP elimination, spore germination on the filter membrane and extracted the lysate for analysis. By filtering 1L, we can achieve sensitivity of 10 CFU/ 100 ml. These results indicate that the system can be utilized for rapid microbiological testing methods requiring high sensitivity.

PM078 Chemical composition and antimycoplasmal activity of a green propolis from Itapará (Paraná State, south Brazil)

Camila Nascimento Araujo[1], Aline Teixeira Amorim[1], Regiane Yatsuda[2], Jorge Timenetsky[1], Guilherme Barreto Campos[2]

[1]ICB-USP - Departamento de Microbiologia, Microbiologia, Sao Paulo, Brazil
[2]Universidade Federal da Bahia (UFBA) - Campus Anísio Teixeira, Microbiologia, 68, Brazil

Background: Propolis is a beehive product containing chiefly resins collected by the insects from plants and beeswax. It acquires sticky texture upon heating. Most propolis types have resinous flavor and some exhibit characteristic smell. Propolis has been used popularly to treat and prevent diseases, as well as to enhance the resistance against infections, presumably with low or no risk of side effects.

Objectives: The objective of the present study was to characterize the chemical constituents of extracts with distinct polarities of green propolis produced in Itapará (Brazil) and evaluate their activity against pathogenic mycoplasmas to humans and domestic animals.

Methods: The chemical constituents of propolis were analyzed using HPLC-DAD-ESI-MS system. Seven mycoplasma strains were used in the antimicrobial assays: *Mycoplasma pneumoniae*, *M. genitalium*, *M. hominis*, *M. penetrans*, *M. gallisepticum*, *M. hyorhinis* and *M. bovis*.

Results: Relatively high contents of drupanin, 2,2-dimethyl chromene-6-propenoic acid, artepillin c and baccharin were detected in the Itapará propolis extracts. The best antibacterial activity results was obtained using extracts A (hexane), B (hexane:ethyl acetate) and C (ethyl acetate) with MICs at 3.9 mg/ml to *M. pneumoniae* and *M. bovis*. However, lower activity against *M. penetrans* was showed by extract A (250 mg/ml) and for *M. genitalium* (250 mg/ml) by extract B. *M. bovis* was resistant to extracts D (ethanol) and E (aqueous). Extracts of the Itapará propolis showed antimicrobial activity against pathogenic mycoplasmas. To the best of our knowledge, this is the first report on the antibacterial activity of green propolis against mycoplasmas species.

PM079 Assay development for the discovery of small-molecule inhibitors of YadA adhesion to collagen

Athanasios Saragliadis[1], Dirk Linke[1]

[1]*University of Oslo, Department of Biosciences, Oslo, Norway*

Background: Bacterial adhesion is the first step of bacterial colonization and an essential part of invasive processes as well as of biofilm formation. The proteinaceous bacterial appendages known as adhesins are attractive target candidates for discovering novel antimicrobial compounds. One family of adhesins exclusive to Gram-negative bacteria, the trimeric autotransporter adhesins (TAAs), are essential for the pathogenesis of a number of *Enterobacteriacae*, including *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* and appear to be important in various stages of pathogenesis in entero- and uropathogenic *E. coli*, *Salmonella* and *Proteus*.

Objectives: We set out to develop scalable assays to measure bacterial adhesion to mammalian extracellular matrix (ECM) proteins, with the aim to perform high-throughput screening for inhibitors. Our model system is the trimeric autotransporter adhesin YadA from *Yersinia enterocolitica* that binds to collagen.

Methods: Using bacterial cells expressing GFP under an inducible promoter and co-expressing the adhesin of choice, we established a 384-well plate-based assay that allowed us to screen 28000 compounds in 8 days (3520 compounds per day). We have collected the essential parameters in assay development and describe how they can be tuned for improved performance.

Results: Out of 28000 compounds, 5 compounds showed significant inhibitory activity, measured as loss of fluorescence compared to control wells. Our assay is easy to scale up and can be adopted to different ECM component/adhesin combinations. Alternatively, bacterial pathogens (harboring deletion mutants of adhesins compared to wildtype) could be used directly in the same assay if they express GFP as a reporter at high levels.

PM080 Unmasking the potential as antibiotic makers of three streptomyces strains isolated in a high-altitude ecosystem in colombia

Linamaria Pintor Escobar[1], María Mercedes Zambrano[2], Lorena T. Fernández-Martínez[1]

[1]*Edge Hill University, Ormskirk, United Kingdom*
[2]*Corporación Corpogen, Bogotá D.C., Colombia*

Background: The current threat of antimicrobial resistance, the surge in antimicrobial compounds rendered obsolete and the slow emergence of new classes of antibiotics have triggered an urgent call for novel alternatives to treat infectious diseases. The vast microbial diversity of unexplored environments and the chemical and structural variety of specialised metabolites within it stand as one of the central points to tackle this challenge.

Objectives: This work aims to explore the potential for antimicrobial compounds in three new Streptomyces strains -Streptomyces sp. CG885, Streptomyces sp. CG893 and Streptomyces sp. CG926- isolated in the Natural National Park Los Nevados (Colombia) which produce active metabolites against several ESKAPE pathogens.

Methods: We constructed a 16S rRNA phylogenetic tree and studied the specialised metabolite potential of these isolates using a genome mining approach to predict the presence of putative Biosynthetic Gene Clusters (BGCs). Findings from this bioinformatic prediction were linked with analysis from active extracts obtained through agar plate extraction to inform prioritization of clusters.

Results: So far, the computational analysis has predicted between 68 to 93 specialised metabolite BGCs for each strain. Interestingly, most of these compounds show less than 15% similarity to any known compound in the database and around 69 clusters seem related to previously uncharacterised RiPPs, NRPS and PKS. Further work will focus on the validation and study of those clusters linked to compounds with potential in clinical development.

PM081 Demonstration of Bacterial Migration due to Medical Device Insertion in a Novel In vitro Urethral Model

Yvonne Cortese[1;2], Victoria Wagner[3], Morgan Tierney[3], David Scully[3], Declan Devine[1], Andy Fogarty[2;4]

[1]*Athlone Institute of Technology, Material Research Institute, Athlone, Ireland*
[2]*Athlone Institute of Technology, Bioscience Research Institute, Athlone, Ireland*
[3]*Teleflex, Reading, PA, United States*
[4]*Athlone Institute of Technology, Life and Physical Sciences, Athlone, Ireland*

Background: Urinary catheters are commonly used medical devices, however throughout their history there has been difficulties in controlling concomitant bacterial infections. Catheter associated-urinary tract infections (CAUTIs) are common hospital-acquired infections. All catheter types and brands are vulnerable to pathogen contamination.

Objectives: There is currently no standard established *in vitro* model to test the efficacy of medical devices, in particular intermittent catheters, to prevent or control introduction/movement of bacteria into the urethra during device insertion. This study aims to address this issue by developing a reproducible agar based *in vitro* urethral model.

Methods: The urethral model consists primarily of a preformed channel within an agar based matrix. The urethral model utilises a specific agar formulation which indicates bacterial growth. The model production and associated methodology are IP protected.

Results: Numerous problems have been encountered during the *in vitro* model development process. Poor visualisation of microbial growth was an initial problem which was solved with a change in media formulation. Temporal contamination was also a significant challenge and was solved by a redesign of the urethra model mould. Condensation and bacterial swarming were also significant issues, affecting primarily the reproducibility of the model. After 6 months of improvements, reproducibility issues have been resolved. The urethra model is now generating reproducible data and is currently under validation with the aim to publish within one month. The model developed at Athlone Institute of Technology aims provide a robust model to aid in the improvement of future urological devices focused toward preventing hospital acquired infections.

PM082 Identification and Characterisation of Novel Antimicrobial-Producing Bacterial from a Thermal Spring Source using Oxford Nanopore Whole Genome Sequencing and Natural Product Chemistry

Tim Walker[1], Paul Stapleton[1], Simon Gibbons[1]

[1]*University College London, UCL School of Pharmacy, London, United Kingdom*

Background: Microbially-derived antimicrobial compounds are a rich source of clinical antibiotic leads. However, discovery rates have declined over the past 40 years due, in part, to high rediscovery rates of known compounds from traditional soil-based screening approaches. Researchers have therefore turned their attention in recent years to previously underexplored environments in the hope that microbes adapted to survive in different environmental conditions may be capable of producing metabolites with novel chemical structures.

Objectives: The aim of this study was to isolate and characterise novel antimicrobial-producing bacterial isolates by sampling a previously underexplored thermal-spring source.

Methods: Bacteria were cultivated and tested for antimicrobial activity using cross-streak assays against *Staphylococcus aureus* and *Escherichia coli.* The genomes of lead isolates were sequenced using Oxford Nanopore and Illumina platforms for phylogenetic characterisation and to identify putative secondary metabolite gene clusters. Antimicrobial compounds were isolated using chromatography and bioactivity-guided isolation.

Results: Two antimicrobial-producing bacteria were isolated which were revealed by 16S rRNA typing to be in the genera *Streptomyces* and *Paenibacillus* respectively. Whole genome orthology analysis and annotation of the *Streptomyces* genome revealed it to be highly related to *Streptomyces canus* and to contain 26 putative secondary metabolite gene clusters - some of which were potentially novel. Investigations of the Streptomyces by natural product chemistry techniques showed the organism to produce multiple antimicrobial compounds effective against methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus*. This study underlines the value of investigating non-traditional habitats in the search for novel antibiotic-producing organisms.

PM083 Outer membrane porin OmpC is a key intrinsic carbapenem resistance determinant in Escherichia coli: a potential new drug target for future antimicrobials

Shiela Chetri[1], Melson Singha[1], Deepshikha Bhowmik[1], Debadatta Dhar[2], Amitabha Bhattacharjee[1]

[1]*ASSAM UNIVERSITY, SILCHAR, Microbiology, Silchar, India*
[2]*Silchar Medical College and Hospital, Silchar, Microbiology, India*

Background: Carbapenem resistance in *Escherichia coli* is often attributed to acquired mechanisms. However, intrinsic mechanisms are mostly over looked and not much research on their response against external antibiotic stress was undertaken. Mechanisms like increased efflux or decreased influx are key factors that offer nonspecific resistance to antibiotics. OmpC and OmpF are two major outer membrane proteins in *E. coli* which serves as a barrier for antibiotics and other toxic agents entering inside the cell.

Objectives: Therefore, current study was designed to investigate the transcriptional response of OmpF and OmpC under concentration gradient carbapenem exposure.

Methods: A total of 96 consecutive, non-duplicate clinical isolate of *E. coli* resistant to at least one of the carbapenem antibiotic were collected from Silchar Medical College and Hospital, Silchar, India. Selected isolates were further subjected to antibiogram profiling. Transcriptional response of OmpF, OmpC and MicF gene was analyzed using quantitative real time PCR detection system with and without differential concentration gradient carbapenem stress.

Results: An elevation in the expression of OmpF gene under concentration gradient imipenem stress from a particular concentration was observed. For OmpC gene a significant decrease in the expression was noticed under concentration gradient imipenem and meropenem stress. The study showed reduction in the expression of OmpC gene against imipenem and meropenem possibly preventing the entry of carbapenem antibiotic inside the cell indicating a possible role in carbapenem resistance. Thus, this porin could be a potential drug target for next generation antimicrobials against multi drug resistant bacteria.

PM084 Iron-oxidation Activity and Growth of Sulfobacillus thermosulfidooxidans and of Alicyclobacillus sp. S09: Potentially Useful for Bioleaching in the Presence of Chloride

Michael Schlomann[1], Ngoc-Dieu Huynh[1], Wolfgang Sand[2], Javier Rivera[3], Stefan Kaschabek[1], Gloria Levicán[3]

[1]TU Bergakademie Freiberg, Interdisciplinary Ecological Center, Freiberg, Germany
[2]Donghua University, College of Environmental Science and Engineering, Shanghai, China
[3]Universidad de Santiago de Chile, Dept. de Biología, Santiago, Chile

Background: Bioleaching applies acidophilic sulfur- and iron-oxidizing microorganisms to extract valuable metals from sulfidic low-grade ores or concentrates. This technology has become an economical alternative to conventional techniques of metal extraction. However, acidophilic microorganisms in general tend to be intolerant to high concentrations of chloride. This restriction hampers the use of sea water and the recycling of raffinate solutions.

Objectives: The study aimed at identifying or obtaining acidophilic bacteria that are able to oxidize iron and bioleach chalcopyrite at high chloride concentrations.

Methods: *Sulfobacillus thermosulfidooxidans* DSM 9293^{T}was grown with ferrous iron as sole inorganic growth substrate in Mackintosh medium pH 1.8, 0.02% yeast extract at 45°C in presence of various NaCl concentrations. Isolation of new strains was performed with Mackintosh medium pH 2.5 at 37°C with 20 g/L (340 mM) NaCl. Leaching experiments with 3% chalcopyrite were performed in modified Mackintosh-artificial seawater pH 2.5 at 37°C.

Results: *Sb. thermosulfidooxidans* tolerated relatively high NaCl concentrations, being able to grow and to oxidize iron in the presence of up to 29 g/L (500 mM) NaCl. This is considerably higher than found for most iron-oxidizers of the genera *Acidithiobacillus* and *Leptospirillum*. Additionally, a new acidophilic isolate, *Alicyclobacillus* sp. S09, was obtained from a tailing near the Spanish coast. It tolerated more than 50 g/L (855 mM) NaCl. In bioleaching experiments in presence of 35 g/L NaCl (600 mM) it showed higher copper extraction than the abiotic control. Thus, bioleaching by *Sb. thermosulfidooxidans* and the *Alicyclobacillus* in presence of NaCl will further be investigated.

PM085 Antimicrobial sensitivity pattern of urine and biofilm in catheterized patients in Asmara, Eritrea

Lia Tekle[1;2;2], Berzelin Haile[3]

[1]Kabalagala, Microbiology, Kampala, Uganda
[1]Kabalagala, Microbiology, Kampala, Uganda
[3]National health Laboratory, Microbiology, Asmara, Eritrea

Background: The biofilm mode of growth is a basic survival strategy implemented by bacteria in a wide range of settings. Bacterial growth on the inner surface of the catheter with biofilm formation is frequent and may occur within days of catheter placement.

Objectives: Investigated the formation of biofilm inside catheter lumen of patients. And also, it assessed the antimicrobial sensitivity pattern of biofilm isolates and compared it with urine isolates.

Methods: Prospective cross-sectional study done in the microbiology department of the national health laboratory, Asmara, Eritrea. Patients who have undergone indwelling catheterization for urinary retaining and some for urinary incontinence has developed biofilm in the inner surface of catheters which could be seen by naked eye was included in this study.

Urine sample was collected in sterile, wide mouth container through suprapubic puncture. A cross section of indwelling catheter containing biofilm seen on naked eye was our second sample. A modified standardized semi-quantitative technique using calibrated bacteriological loop of urine was performed to transfer the 10 ul of sample on Nutrient agar. After allowing the urine to be absorbed into the agar, the plates were then inverted and incubated aerobically at 37°C for 24 hours. Antimicrobial sensitivity test was carried out by disc diffusion technique using Muller Hinton Agar.

Results: A significant correlation between biofilm formation and multidrug resistance was observed. Since 15 samples from biofilm showed resistance to all tested antibiotics and also since 42.15% of the biofilm strains showed multidrug resistance. *E.coli* was the most frequently isolated organism.

PM086 Antibacterial Activities of Plant Extracts against Bovine Mastitis-Associated Bacteria

Chadanong Chaiwut[1], Maytiya Konkit[1], Hataichanok Pandith[1], Sakunnee Bovonsombut[1], Thararat Chitov[1]

[1]*Chiang Mai University, Department of Biology, Chiang Mai, Thailand*

Background: Bovine mastitis is one of the major problems in dairy farms. It is caused mainly by bacterial infection of the udders, resulting in milk of lower quality. In addition, antibiotic treatment of mastitis can leave antibiotic residues in milk, and can increase dairy cattle reservoir for antibiotic resistant bacteria in the food chain.

Objectives: The objectives of this work were to conduct an in-depth investigation of persistence of mastitis-associated bacteria in a farm that was being in transition to organic farming and to determine plant extracts that can potentially be used instead of antibiotics for mastitis control.

Methods: Bacteria were isolated from raw milk from cows with subclinical mastitis in 3 consecutive years, 2016 to 2018. The isolates were identified and their relatedness were analysed using genetic analysis. Their susceptibilities to antibiotics and plant extracts were determined. The plant extracts included water and 70% ethanolic extracts of turmeric (*Curcuma longa*), mango ginger (*Curcuma mangga*), ginger (*Zingiber officinale*), cassumunar ginger (*Zingiber cassumunar*), betel leaf (*Piper betle*), neem (*Azadirachta indica*), and sappan wood (*Caesalpinia sappan*).

Results: The major bacteria associated with subclinical mastitis in this farm were *Staphylococcus* and *Streptococcus*. Many *Staphylococcus* species, such as *S. saprophyticus* subsp. *bovis*, *S. Simulans*, and *S. chromogenes* were found to be persistent in the farm. While some mastitis-associated species were resistant to some antibiotics, they were all susceptible to extracts of sappan wood, mango ginger, betel leaf, and neem. These plant extracts can be developed into medicinal products for bovine mastitis control.

PM087 Optimisation of lignocellulose degrading enzyme production from white rot fungi

Linda Mezule[1], Ieva Berzina[1]

[1]*Riga Technical University, Rīga, Latvia*

Background: Already for many years it has been recognized that lignocellulosic biomass has a high potential to be used as a source for energy and valuable chemical production. At the same time practical application has been limited due to either high conversion costs, low efficiency or environmental issues (unsustainable technologies for conversion like concentrated acid hydrolysis). From all technologies, biological approaches have been recognized as the most sustainable ones, however, the need for specific lignocellulose degrading enzymes and slow conversion rates, have increased the production costs, thus, making the process cost-inefficient.

Objectives: The need for new enzyme formulations that have lower production costs, compatibility with current commercial products (currently being produced in limited amount of big companies) and better use conditions is one of the acute issues and has been the emphasis of this research.

Methods: Laboratory and semi pilot scale tests have been performed with various fungal cultures, enzyme extracts and production conditions have been characterized and evaluated on various biomass products, e.g., hay, straw and biomass waste.

Results: Our research has shown that enzyme products obtained from white rot fungi and other wood decay fungi can demonstrate comparable conversion efficiency in less than 30 hours at mild environmental conditions. Moreover, the latest research has demonstrated that the production is less sensitive than previously described, e.g., in terms of fungal growth and incubation conditions.

Acknowledgement: This work has been supported by IPP3: INNO INDIGO Project B-LIQ, No. ES/RTD/2017/18.

PM088 Construction of leaderless bacteriocin producing phage

Yoshimitsu Masuda[1], Shun Kawabata[1], Ken-ichi Honjoh[1], Takahisa Miyamoto[1]

[1]Kyushu University, Department of Bioschence and Biotechnology, Fuculty of Agriculture, Fukuoka-shi, Japan

Background: Due to antibacterial activities against pathogenic bacteria by bacterial lysis, lytic (bacterial) phages were used as effective treatment for bacterial infections in human before antibiotic era and have recently re-emerged as promising alternative for sanitizing food pathogens and spoiling bacteria in foods. Leaderless bacteriocins (LLB) are very simple bacteriocins produced by gram positive bacteria. LLB does not possess N-terminal leader peptide in precursor, which means LLB become active immediately after translation. This simple structure allows us easy heterologous expression with introducing only its structure gene into host strains.

Objectives: In order to enhance antibacterial activity of phage, this study aims to construct the LLB producing phage (LLB-phage). Especially, when Escherichia coli phages such as T7 phage are used as hosts, LLB-phage will kill not only host E. coli by infection, but other gram-positive neighbors by LLB simultaneously.

Methods: LLB, Lacticin Q (LnqQ) structure gene lnqQ was genetically integrated into lytic phage (T7 phage) by homologous recombination in E. coli DH5α containing lnqQ in non-expressing plasmid. Mutated lnqQ-T7-phage was screened by CRISPR-Cas3 system in E. coli BL21 (DE3) from phage mix solutions after infection and lysis with E. coli DH5α. Efficiencies of homologous recombination and CRISPR-Cas3 screening were evaluated by real time qPCR targeting lnqQ insertion point of T7 phage.

Results: The plasmid for homologous recombination (plnqQ) and for CRISPR-Cas3 system (pSp24031 and pCas) were successfully constructed with pET-UK and pACYC-duet1. Homologous recombination and CRISPR-Cas3 system were evaluated by real time qPCR and have been being improved.

PM089 Cross-kingdom interaction between Candida albicans and Streptococcus oralis mediated by streptococcal glucosyltransferase R

Joao Gabriel Silva Souza[1;2], Martinna Bertolini[2], Angela Thompson[2], Jillian Mansfield[3], Valentim Barão[1], Margaret Vickerman[3], Anna Dongari-Bagtzoglou[2]

[1]*Piracicaba Dental School, University of Campinas, Piracicaba, Brazil*
[2]*University of Connecticut Health Center, Farmington, United States*
[3]*University at Buffalo, School of Dental Medicine, Buffalo, United States*

Background: Streptococcal glucosyltransferases (Gtf) synthesize α-glucan exopolymers which contribute to biofilm matrix. *Streptococcus oralis* interacts with the opportunistic pathogen *Candida albicans* to form hypervirulent oral biofilms. *S. oralis* 34 has a single *gtf* structural gene (*gtfR*). However, the role of *gtfR* in single and mixed species biofilms with *C. albicans* has never been examined.

Objectives: To explore the role of gtfR in biofilm development.

Methods: A *gtfR* deletion mutant in *S. oralis* 34 was constructed by allelic replacement of an internal segment of *gtfR* with an *aad9* gene encoding spectinomycin resistance. A complemented strain with a plasmid-borne copy of *gtfR* was used as control. *C. albicans* strain SC5314, which forms robust biofilms with *S. oralis* 34, was used. The role of the glucan binding domain of *GtfR* in substratum adhesion was tested by generating recombinant protein (rGBD). Biofilms grown on poly-L-lysine coated slides, polystyrene plates, titanium disks or oral mucosal organotypic constructs were analyzed by confocal microscopy, viable counts and RT-qPCR.

Results: In single species biofilms S. oralis parental and complemented strains expressing gtfR had increased biovolume due to α-glucan matrix synthesis, but no difference in bacterial biomass, compared with the gtfR mutant. In contrast, in biofilms with C. albicans, S. oralis encoding gtfR showed increased bacterial growth on all surfaces. On titanium surfaces gtfR had a positive influence on both fungal and bacterial biomass. RT-qPCR indicated up-regulation of gtfR expression in mixed compared to single biofilms on titanium. rGBD did not influence microbial adhesion to any substratum.

PM090 High stable-emulsification biosurfactant formation from yeasts isolated from Raphia africana and Elaeis guineensis

Ijeoma Nwaguma[1], Chioma Blaise Chikere[2], Gideon Chijioke okpokwasili[2]

[1]*ACE-CEFOR, University of Port Harcourt, Nigeria, Microbiology, Port Harcourt, Nigeria*
[2]*University of Port Harcourt, Microbiology, Port Harcourt, Nigeria*

Background: Yeasts are preferred sources of biosurfactant production than bacteria and mould because of their capacity to produce biosurfactant in high concentration and their "generally regarded as safe" (GRAS) status.

Objectives: This study investigated the production of stable emulsification by yeasts isolated from saps of *Elaeis guineensis* and *Raphia africana.*

Methods: Chemical composition of *Raphia africana* and *Elaeis guineensis* saps were determined. Biosurfactant production was screened using emulsification index (E_{24}), oil spreading, and tilted glass slide. Emulsification test was carried out for 15 days using kerosene.

Results: Chemical analyses of saps from *Elaeis guineensis* and *Raphia africana* revealed reducing sugar content of 2.099 and 1.09 mg/mL, respectively. Isolates SA2 and SB6 showed the most profound emulsification stability over a 15-day period out of the 8 positive biosurfactant-producing yeasts. Isolates SA2 from *Elaeis guineensis* and SB6 from *Raphia africana* retained 92.2% (62.5% to 57.6%) and 84.5% (61.2% to 51.7%), respectively within 15-day period. Based on biochemical characteristics, SB6 (germ tube positive) and SA2 were placed on the probable genera *Candida* and *Saccharomyces*, respectively. Extraction and PCR analysis of internally transcribed spacer (ITS) genes of both SA2 and SB6 generated 530 bp amplicons. Isolates SA2 and SB6 preferred olive oil as substrate for biosurfactant production. The crude biosurfactant produced by SA2 and SB6 displaced a thin film of crude oil on petri dish by 55 and 48 mm, respectively. This study demonstrated that saps from *Elaeis guineensis* and *Raphia africana* were suitable sources of biosurfactant-producing yeasts that formed stable emulsions.

PM091 Evaluation of the presence of Helicobacter pylori strains possessing cagA in patients with stomach cancer and gastritis

Saeed Shams[1], Fateme Sadat Mirtalebi Roknabadi[2], Mahya Teymoori[2], Ahmad Hormati[3], Mahdieh Ghoddoosi[4]

[1]*Qom University of Medical Sciences, Cellular and Molecular Research Center, Qom, Iran*
[2]*Islamic Azad University, Qom Branch, Department of Genetics, Qom, Iran*
[3]*Qom University of Medical Sciences, Gastroenterology and Hepatology Disease Research Center, Qom, Iran*
[4]*Qom University of Medical Sciences, Pathology laboratory, Qom, Iran*

Background: *Helicobacter* pylori (*H. pylori*) is the causative agent in the development of the gastrointestinal diseases. Cytotoxin-associated gene A (*cagA*) is one of the virulence factors of *H. pylori*. $CagA^+$ strain is associated with increased risk of gastric adenocarcinoma.

Objectives: The aim of the study was to evaluate the presence of the *cagA* gene two groups of patients with stomach cancer and gastritis, as the first study in our area.

Methods: First, the written informed consent was provided by the participants referred to our central hospital. The presence of the *H. pylori* was evaluated by urease test and Giemsa staining in gastric biopsy and pathologic samples, respectively. To confirm, conventional PCR was also done at conserved regions of the 16S rRNA gene in all samples. Finally, the presence of the *cagA* gene was tested using specific primers.

Results: Among patients, 80 patients were positive for *H. pylori* (40 cancerous and 40 non-cancerous). Mean±SD age of *H. pylori* positive people was 45.36±14.73 years. In non-cancerous patients, the most common complaint was stomach pain (32 cases), while dyspepsia (48.8%) and duodenal ulcer (27.9%) were the most common endoscopic findings. In cancerous patients, intestinal-type adenocarcinoma was the most pathologic results (85%). The prevalence of the *cagA* gene among cancerous and non-cancerous patients was 27.5% and 32.5%, respectively. According to the obtained data, the rate of *cagA* gene was relatively high both groups in our geographical location, but no significant difference was seen between stomach cancer and the presence of the *cagA* gene.

PM092 Characterization of the polysaccharides from a marine diatom

Su-Yuan Lai[1], Min-Ying Wang[2], Hsing-Li Lai[2], Ping-Ting Lin[2]

[1]*Central Taiwan University of Science and Technology, Department of Food Science and Technology, Taiwan*
[2]*National Chung Hsing University, Graduate Institute of Biotechnology, Taiwan*

Background: Polysaccharides, also known as glycans, are the most abundant form of carbohydrate materials in marine algae. A semi-continuous system, *i. e.,* with fixed harvesting frequency, was employed to cultivate a local-isolated marine diatom *Halamphora* sp. for the production of cell mass and polysaccharides.

Objectives: To characterize the polysaccharides purified from diatom *Halamphora* sp.

Methods: Three cultivation sets (set 1 – set 3) were performed according to different harvesting volumes (2, 1 and 3 L) per 3 days. To test whether the addition of Na_2CO_3 can increase the cell mass or polysaccharide production, three more cultivation sets (set 4 – set 6) were performed with the addition of Na_2CO_3. The polysaccharides produced by diatoms are divided into intracellular, adhesive and extracellular polysaccharides. Among them, soluble intracellular carbohydrates were applied to an anion-exchange resin for polysaccharide characterization. Following anion-exchange chromatography, three soluble intracellular polysaccharides extracted from *Halamphora* sp. were eluated at different NaCl concentrations (0.1, 0.5 and 1M).

Results: A high average cell concentration (0.61 g/L) and a high average specific polysaccharide production (44.68 mg/g dried cell weight) were found for the cultures in set 5, *i. e.,* with the addition of Na_2CO_3 and a harvesting volume of 2 L per 3 days. Based on uronic acid detection, FT-IR and monosaccharide composition analysis, the polysaccharide eluated by 1M NaCl is a sulfated acid polysaccharide, mainly composed of fucose and galactose. The polysaccharide eluated by 0.1M NaCl is mainly composed of glucose, fucose and galactose and is a neutral polysaccharide.

PM093 Ecology, dynamics and future prospects of lignocellulose-degrading microbial consortia

Diego Jimenez[1]

[1]*Universidad de los Andes, Biological Sciences, Bogota, Colombia*

Background: Agricultural residues are key sources for commodity chemicals production. However, an efficient saccharification of plant biomass is still a challenge in biorefineries. The use of enzyme cocktails produced from lignocellulose-degrading microbial consortia is a promising approach to optimize this process. Nevertheless, the proper use of these consortia depends on a sound understanding of the ecology, dynamics and their enzymatic mechanisms

Objectives: In a recently published manuscript, we have explored the temporal expression profile of carbohydrate-active enzymes in a synthetic bacterial consortium growing on sugar cane bagasse. Moreover, within this topic, I would like to show new ecological and biotechnological questions or prospects. For instance, i) it is possible to build a minimal and versatile consortium using two approaches of selection (dilution-to-stimulation and dilution-to-extinction)? ii) how design an effective enzyme cocktail using a consortial secretome plus specific fungal enzymes?, and iii) can we evaluate stochasticity events within the selection process of these consortia?

Methods: Metatranscriptomic-based approach

Results: In our synthetic bacterial consortium, we observed a clear successional profile, where endoglucanases and endoxylanases were significantly expressed at 12 hours of growth. In contrast, enzymes acting on external-side linkages of plant polysaccharides, as exoglucanases and alpha-arabinosidases/beta-xylosidases, were highly expressed after 48 hours. Here, *Paenibacillus*, *Brevundimonas* and *Chryseobacterium* were the most important contributors, whereas *Stenotrophomonas* was highly active at the end of the culture without contributing largely to the expression of lignocellulolytic enzymes.

PM094 Evaluation of the tolerance and the biotransformation of ferulic acid by Klebsiella pneumoniae TD 4.7

Eleni Gomes[1], Maite Bernardo Correia dos Santos[1], Guillermo Ladino-Orjuela[1], Roberto Da Silva[2], Mauricio Boscolo[2]

[1]Sao Paulo State University-Unesp, Biology, São Jose do Rio Preto, Brazil
[2]Sao Paulo State University-Unesp, Chemistry, São Jose do Rio Preto, Brazil

Background: Lignocellulosic biomass can be used to produce sugars from cellulose hemicellulose and aromatic compounds derived from lignin. One of the aromatics compounds released during the deconstruction of lignocellulose is ferulic acid whose biotransformation can result in the generation of important flavors products

Objectives: This study looks at the bioconversion of ferulic acid to 4-vinylguaiacol by *Klebsiella pneumoniae* TD 4.7

Methods: The biotransformation of commercial ferulic acid into 4-vinylguaiacol was studied in a synthetic liquid medium containing the ferulic acid at an initial concentration of 300 mg L^{-1} .Following on, the bacterium was used to convert the ferulic acid produced by the alkaline hydrolysis of the sugar cane bagasse, also at a concentration of 300 mg $L^{-1.}$

Results: The biotransformation of commercial ferulic acid into 4-vinylguaiacol at an initial concentration of 300 mg L^{-1} was 32.4% of . Following on, the bacterium was used to convert the ferulic acid produced by the alkaline hydrolysis of the sugar cane bagasse, also at a concentration of 300 mg L^{-1}. After 32 hours of cultivation, the yield of 4-vinylguaiacol was 1.3 mmol L^{-1} corresponding to a conversion of 81.7% of the ferulic acid. The results showed that the strain decarboxylated the ferulic acid to 4-vinylguaiacol and the presence of an active cell associated ferulic acid decarboxylase. These results show promise for the use of this enzyme in the production of 4-vinyl guaiacol

PM095 Isolation and identification of the thermostable gum arabic-digesting enzymes producing actinomycetes

Chaohsun Yang[1], Yu-Chun Huang[1], Wei-Lin Chen[1], Chui-Li Lim[1]

[1]*Providence University, Department of Cosmetic Science, Taichung , Taiwan*

Background: Gum arabic, which is a mixture of polysaccharide and glycoprotein, is primarily used as an emulsifying stabilizer in the food industry. It has also been shown that the modification of gum arabic may alter the functional properties. However, little of the research in enzymatic treatments of gum arabic has been published.

Objectives: The purpose of this study is to screen the potent strains processed of thermostable gum arabic-digesting enzymes from seventy domestic thermophilic actinomycetes, which are isolated from the compost in Taiwan.

Methods: The gum arabic-digesting enzymes producing strains were screened on a CYC agar medium supplemented with 1% gum arabic. The formation of a clearing zone around the point of inoculation indicated gum arabic-digesting enzymes production. The selected strain was cultured in a 50 mL of gum arabic-CYC medium in a 500 mL Hinton flask. For gum arabic-digesting enzymes assay, the reaction mixture contained gum arabic and culture supernatant to a final volume of 1.0 mL and incubated for 6 h at 50°C. The enzyme activity was determined by measuring the release of reducing sugar.

Results: Among 70 strains, strain No. 1511 and No. 23-3 had the best gum arabic degrading abilities. When the biological characteristics were compared with descriptions in Bergey' s Manual of Determinative Bacteriology and the 16S rDNA sequences with the GenBank database, the greatest similarities were found with *Thermobifida fusca* and *Thermomonospora curvata*. Therefore, the selected thermophilic actinomycetes strains were identified as *Thermobifida fusca* PU-1511 and *Thermomonospora curvata* PU-23-2.

PM096 A Decision Equivalence Comparability Study for a Rapid Sterility System vs. Compendial Methods

Carol Iversen[1], Asha Rani Ram[2]

[1]*Merck BioReliance, Global Operational Development Services, Glasgow, United Kingdom*
[2]*Merck BioReliance, Microbiology Operations Department, Glasgow, United Kingdom*

Background: The traditional 14-day Sterility test is difficult to apply to biopharmaceutical treatments (somatic cell and gene therapies), due to the test volume and turn-round-time. These materials are manufactured as small volume, short shelf-life products which often need to be administered before sterility test results are available. Rapid sterility test methods are available offering greater sensitivity and a faster turn-round-time; however these methods need to give equivalent decision results to the traditional method.

Objectives: This study compared the performance of the BacT/ALERT 3D Rapid Microbial Detection System against the standard harmonized USP<71> , EP2.6.1 and JP4.06 sterility test methodology. The method performance for use of this RMM according to EP2.6.27 was also evaluated.

Methods: Test organisms comprising all the named reference strains in the compendial methods were diluted to inoculation levels of 40, 4 and 0.4 CFU. The respective media for each method were inoculated with the appropriate strains (n=6 per dilution). For EP2.6.27 evaluation cellular material (C8166 cells) was used as representative test material. The Boolean data generated from each methodology (presence/absence) was used to determine the Limit of Detection (LOD) for each organism, additionally the Relative LOD (RLOD) was determined where the RMM was compared to the compendial method.

Results: Using the BacT/ALERT 3D System the LOD50% for all EP2.6.27 reference organisms was <10 CFU and the LOD50% for all EP2.6.1 reference organisms was <1 CFU. All organisms were detectable within a maximum of 7 days. No significant difference was found between the compendial method and the RMM.

PM097 Probiotic characteristics of Lactobacillus stains isolated from a dog feces

Min Young Jung[1], Jung-Ae Kim[1], Dae-Hyuk Kim[1;2], Yangseon Kim[1]

[1]Center for Industrialization of Agricultural and Livestock Microorganisms, Department of Research and Development, Jeongeup-si, Korea, Rep. of South
[2]Chonbuk National University, Department of Molecular Biology, Jeonju-si, Korea, Rep. of South

Background: Probiotics are known to control the balance of gut microbiota and beneficial to host animal health in multiple ways. Lactic acid bacteria (LAB) are well known for their probiotic potential.

Objectives: The present study evaluated the probiotic characteristics of microbial strains for potential use as feed additives.

Methods: The probiotic function such as acid and bile resistant ability, antibiotics susceptibility of LABs, *Lactobacillus paracasei* CACC566 and *L. plantarum* CACC558 isolated from canine feces were investigated. In addition, antimicrobial activity of CACC566 and CACC558 were tested against livestock pathogens, *Escherichia coli*, *Salmonella* spp. *Yesinia* spp. and *Candida* spp. To gain insight into its probiotics characteristics in interactions with the host, whole genome sequencing, and comparative genomic analysis of CACC566 was carried out.

Results: Two strains exhibited antibiosis, antibiotic activity, acid and bile tolerance and relative cell adhesion to HT-29 monolayer cell line. The complete genome of strain CACC566 containing 3.24Mb with a G+C content of 46.3 mol% possessed the factors beneficial to mammal health when consumed as feed, proteins related to mucosal surface adhesion, acid and bile acid-associated genes. The results from *In vitro* assays and predictive gene analysis support our comprehensive understanding for potential food and immune industrial applications of *Lactobacillus* sp. CACC566 and CACC558.

PM098 gc-ms and 1h-nmr-based metabolomic evaluation of a petroleum polluted farmland at ngia ama, niger delta

Emmanuel Fenibo[1], Chioma Blaise Chikere[2], Khayalethu Nthushelo[3], Onyewhuchi Akaranta[4]

[1]*world bank africa centre of excellence, centre for oilfield chemical research, environmental microbiology, Port Harcourt, Nigeria*
[2]*University of Port Harcourt, Microbiology, Port Harcourt, Nigeria*
[3]*university of south africa, Agriculture and animal science, Johannesburg, South Africa*
[4]*University of Port Harcourt, Department of Pure and Applied Chemistry, Port Harcourt, Nigeria*

Background: Ngia Ama (an island) has been hitherto used for artisanal refining activities. The chronically polluted areas have no green vegetation. For obvious reasons *in situ* bioremediation commenced 10 months ago and was monitored with omics techniques including metabolomics. Metabolomics is a post-genomic tool with immense and versatile value that reveals unknown biochemical modes of actions of environmental stressors and how microbes respond to these pollutants.

Objectives: The aim of this study was to elucidate the metabolomics fingerprints as biomarkers of *in situ* bioattenuation that will support amenability of site to bioremediation

Methods: A 4-point composite soil sample was collected at Ngia Ama (4.7947°N, 6.6831°E) with 10-year pollution history. Total petroleum hydrocarbons (TPH) was determined using gas chromatography-mass spectrometry (GC-MS). Secondary metabolites were identified by GC-MS and proton nuclear magnetic resonance (^{1}H-NMR). A web-based tool (the chemical translation service), software (MetaboAnalyst 4, NMRshiftDB2) and databases (Kyoto encyclopedia of genes and genome [KEGG], MetLin, MetaCyc) were used to analyze the GC-MS and ^{1}H-NMR data with a view to elucidating the degradation metabolites, pathways and extant hydrocarbon degrading microorganisms.

Results: Pre-remediation extractable-TPH was 490,630 ppm which far exceeded Nigerian Department of Petroleum Resources' intervention limit (5000 ppm of soil). Thirty five degradation pathways for aromatic hydrocarbons, carbazole, long-chain alkanes and chloro-hydrocarbons with associated microorganisms (*Pseudomonas*, *Burkholderia*, *Sphingomonas* and *Bacillus* spp.) were identified as key drivers of pollutants attenuation. The preliminary metabolomic investigation shows that despite the high level of hydrocarbon contamination (with heavy-end fractions) in the farmland, bioattenuation is evidently taking place.

PM099 Galleria mellonella as a Novel Drug Repurposing platform

Olivia Champion[1], Clio Andreae[2], Nicola Senior[1], Vanessa Francis[1], Amy Housden[1], Claire Richards[2], Richard Titball[1]

[1]BioSystems Technology, University of Exeter, BioSciences, Exeter, United Kingdom
[2]Charles River, 47-48 Martingale Way, Portishead, United Kingdom , Bristol, United Kingdom

Background: Drug repurposing can reduce the time and costs of drug development. In this project we assessed the feasibility of using *G. mellonella* (TruLarv) as a model for drug screening and re-purposing. A *G. mellonella* model would transform drug development pipelines by enabling *in vivo* screening of large panels of re-purposing candidates at an early stage.

Objectives: A panel of 30 immunosuppressive, re-purposed anti-inflammatory and immune system drugs were screened for direct antimicrobial activity against *Burkholderia thailandensis* and for toxicity towards TruLarv. Candidates which lacked antimicrobial activity or toxicity were screened for their ability to modulate an infection of TruLarv with *B. thailandensis*. The change in disease severity was measured when drugs (at <10 x the scaled human dose) were co-administered with an otherwise sub-lethal dose of *B. thailandensis*.

Methods: Drug candidates were tested for toxicity and assessed in an immunomodulation assay. Doses of drugs at greater than 10x the scaled human dose were administered with *B. thailandensis* and changes in disease severity were observed, compared to controls.

Results: Drug re-purposing has the potential to reduce both the time and costs associated with drug development. Here we show that an immuno-modulation assay using TruLarv can provide meaningful assessments of the immunosuppressive activity of drugs. Our results also show that the innate immune system in *G. mellonella* plays a key role in controlling infection and *G. mellonella* can be used as a novel *in vivo* drug re-purposing platform.

PM100 In vitro antifungal activity of Pseudomonas synxantha against Monilinia laxa

Tamara Janakiev[2], Nikola Unković[1], Ivica Dimkić[2], Milica Ljaljević-Grbić[1], Slaviša Stanković[2], Tanja Berić[2]

[1]*Faculty of Biology, University of Belgrade, Department of Micology, Belgrade, Serbia*
[2]*Faculty of Biology, University of Belgrade, Department of Microbiology, Belgrade, Serbia*

Background: There is globally recognized necessity to find safe alternative for pesticide use. Using microorganisms with antagonistic activity against pathogens is one of the biocontrol strategies. *Pseudomonas synxantha* belongs to the *P. fluorescens* group comprising many members with biocontrol abilities. *Monilinia laxa* is fungal causative pathogen of brown rot disease of stone fruits.

Objectives: Evaluation of an isolate of *Pseudomonas synxantha* for its biocontrol properties.

Methods: The antifungal activity of *P. synxantha* isolate (P4/16_1) was tested in initial screening on 11 *M. laxa* isolates and in dual culture assay on three selected *M. laxa* isolates. The antagonistic effect of volatile organic compound from *P. synxantha* was tested in sealed dishes bioassay. The non-volatile antifungal compounds were isolated by benzene extraction of 5 days old *P. synxantha* culture in minimal salt medium. The benzene extract dissolved in methanol was tested on three selected *M. laxa* isolates and inhibition of radial growth (PIRG %) was calculated.

Results: In initial screening *P. synxantha* had activity on all tested *M. laxa* isolates. The antagonistic activity against selected *M. laxa* isolates in dual culture assay was in range from 80-87.5% of growth inhibition. The benzene extract exhibited 57.2-63.04% radial inhibition of mycelial growth. The biocontrol activity of this isolate is partly due to the production of volatile compounds that decrease the growth of the tested *M. laxa* between 15.94 and 29.58% after 14 days of incubation. Isolate *P. synxantha* showed promising potential for biocontrol of brown rot disease causing pathogen and should be investigated further.

PM101 Non-destructive fast identification of bacterial colonies through elastic light scattering

Pierre R. Marcoux[1], Emmanuelle Schultz[2], Valentin Genuer[2], Olivier Gal[3], Chakib Belafdil[3], Damien Decq[2], Max Maurin[4], Sophie Morales[2]

[1]Cea Leti, Division microtechnologies for Biology and Healthcare, Grenoble, France
[2]Cea Leti, Division microtechnologies for biology and healthcare, Grenoble, France
[3]Cea List, Laboratory for Data Analysis and Systems' Intelligence, Palaiseau, France
[4]Centre Hospitalier Universitaire de Grenoble, Laboratoire de Bactériologie-Hygiène Hospitalière Institut de Biologie et de Pathologie , La Tronche, France

Background: The development of label-free and non-destructive methods for the rapid identification of pathogenic bacteria is a major step towards automated clinical diagnosis of infectious diseases. Elastic light scattering (ELS) is an optical method for identification of bacterial colonies directly on agar media. It is an attractive solution, combining simple optical instrumentation, no need for sample preparation or labelling. ELS yields many photons, which allows much shorter acquisition times than other optical identification techniques such as Raman and infrared spectroscopies. As for other phenotypic identification methods, such as MALDI-TOF, elastic scattering relies on a database and machine learning algorithms.

Objectives: We have developed ELS for clinical diagnosis, starting with *Staphylococcus aureus* screening. Our goal is to bring a result (positive/negative) after only 6 h of growth on specific agar medium. Our system was used to collect a database of 38 strains of *S. aureus* and other *Staphyloccocus* species (5459 images) on ChromID agar plates.

Methods: A visible laser targets the colony (30-500 µm) to be identified, then the resulting scattering pattern is recorded with a camera over a short time (< 1s). Finally this image is analyzed through pattern recognition algorithms that yield in a few seconds the most probable identity for the probed colony.

Results: The best correct-identification rate between *S. aureus* and other staphylococci (94.7%) has been obtained using a support vector machine classifier trained on a combination of Fourier-Bessel moments and Local-Binary-Patterns extracted features. This result was obtained on microcolonies having 6h of incubation only.

PM102 Magnetotactic bacteria as microrobots for cancer treatment

David Gandia[1], Lucia Gandarias[2;3], Asier Galicia[1;3], Alfredo Garcia-Arribas[1;3], Iñaki Orue[4], Ana Garcia-Prieto[1;5], Javier Alonso[1;6], Alicia Muela[1;2], M. Luisa Fernandez-Gubieda[1;3]

[1]*Basque Center for Materials, Applications and Nanostructures, Leioa, Spain*
[2]*Universidad del País Vasco, Immunology, Microbiology and Parasitology, Leioa, Spain*
[3]*Universidad del País Vasco, Electricity and Electronics, Leioa, Spain*
[4]*Universidad del País Vasco, SGIker, Leioa, Spain*
[5]*Universidad del País Vasco, Applied Physics I, Bilbao, Spain*
[6]*Universidad de Cantabria, CITIMAC, Santander, Spain*

Background: One of the most interesting approaches for cancer therapy is devising micro-robots capable of targeting and destroying cancer cells. To this respect, it has been proposed to take advantage of the innate capacities of biological entities such as virus or bacteria to interact with other cells and organisms. Magnetotactic bacteria (MTB) are aquatic microorganisms that swim along the Earth's magnetic field lines towards low oxygen regions, using a chain of magnetic nanoparticles (called magnetosomes) as compass needle. This feature is known as magnetotaxis. The existence of the magnetosome chain allows detecting, manipulating and guiding externally the bacteria, making them ideal candidates to be used as cancer treatment biorobots.

Objectives: Our aim is to design a system that allows us to observe and guide the magnetotactic bacteria, and also to analyze their movement.

Methods: We observe and quantify the swimming behavior of *Magnetospirillum gryphiswaldense* in a polydimethylsiloxane (PDMS) microfluidic device enclosing microchannels 100 μm in height, under the uniform magnetic field of 10 mT created by two permanent magnets. We use an optical microscope able to capture images at 25 frames/s and custom software to analize these images.

Results: It is revealed that magnetotaxis enables the directed motion of multiple MTB along predefined paths. Two groups of bacteria with different velocities that fit a Gaussian distribution are diferentiated. The average speeds of both groups are 5 ± 1 μm/s and 43 ± 9 μm/s. The first one corresponds to 30% of non motile bacteria.

PM103 4-Ethylphenol effect on the growth and fermentation of an industrial strain of Saccharomyces cerevisiae

Elizabete A. Covre[1], Reinaldo G. Bastos[1], Sandra Regina Ceccato-Antonini[1]

[1]*UFSCar, DTAiSER, Araras, Brazil*

Background: Ethanol production in Brazil is carried out using non-sterile wort which results in decrease in the process efficiency by the action of contaminating microorganisms. *Dekkera bruxellensis* is capable of producing 4-ethylphenol (EP) from p-coumaric acid in sugarcane worts, but while in wine high EP concentrations are associated with the product deterioration, in the bioethanol industry the effect of EP is not known.

Objectives: To evaluate the effects of EP on *Saccharomyces cerevisiae* (Sc) growth and fermentation under the influence of pH, ethanol concentration and sucrose in YPD-based medium.

Methods: Tests were carried out with different pH (2; 2,5; 3; 3,5; 4) and concentrations of ethanol (0, 1.5, 3, 4.5 and 6%), EP (5; 7.5; 10; 12.5; 15 mg/L) and sucrose (1; 5, 9, 13,17%), using a central compound rotational design to verify the effect of the variables on μ_{max} of a Sc industrial strain. The effect of EP added at the start and after 4h of fermentation was also evaluated.

Results: Values of 4.0, 6%, 15 mg/L and 17% for pH, ethanol, EP and sucrose concentrations, respectively, negatively influenced $\mu_{max.}$ The effect of EP concentration is linked to the ethanol concentration in the medium and it is capable of affecting Sc growth when the concentrations of the other parameters are not suitable for the yeast viability. The addition of EP (10 and 20 mg/L) at the start and after 4 h of fermentation decreased the ethanol production and the number of Sc cells in 12 h of fermentation.

PM104 First detection of FOX-1 AmpC genes in Escherichia coli of Abattoir origin in Abakaliki, Nigeria

Chika Ejikeugwu[1;2], Charles Esimone[3], Ifeanyichukwu Iroha[1;1], Michael Adikwu[3]

[1]Ebonyi State University, Abakaliki, Nigeria
[2]Ebonyi State University, Department of Applied Microbiology, Abakaliki, Nigeria
[3]Nnamdi Azikiwe University, School Of Pharmaceutical Sciences, Agulu., Department of Pharmaceutical Microbiology, Agulu, Nigeria

Background: The unrestricted usage of antibiotics for propagation of animals in this part of the world contributes to the evolution of drug resistant microbes.

Objectives: This study was aimed at detecting the prevalence of AmpC β-lactamase genes from abattoir samples.

Methods: The production of AmpC β-lactamase was phenotypically carried out using the cefoxitin-cloxacillin double-disk synergy test (CC-DDST) and FOX-1 AmpC genes was detected in the *E. coli* isolates using multiplex PCR technique.

Results: A total of 48 *E. coli* isolates was recovered from the anal swabs of cow, and only 35 (72.9 %) isolates of *E. coli* was positive for the production of β-lactamase. Notably, high percentages of resistance to cefoxitin (91.7 %), ceftriaxone (83.3 %), imipenem (85.4 %), ceftazidime (87.5 %), ofloxacin (81.3 %) and gentamicin (85.4 %) were found in the *E. coli* isolates. FOX-1 genes was detected in 3 (6.3) isolates of *E. coli* out of the 48 *E. coli* isolates that was used in this study. This study shows that abattoir represent a major reservoir of resistance genes especially AmpC β-lactamase genes; and this could serve as route for the dissemination of multidrug resistant bacteria in the community. Thus, the molecular identification of drug resistant genes is vital for a reliable epidemiological investigation and the forestalling of the emergence and spread of these organisms through the food chain in this region.

PM106 Metabolomic analysis of PkaE, A Serine/Threonine protein kinase from Streptomyces coelicolor A3 (2) linked to choline degradation

Toshiyuki Hirakata[1], Hiroaki Urabe[2], Takashi Sugita[1]

[1]*Meiji Pharmaceutical University, Department of Microbiology, Kiyose, Tokyo, Japan*
[2]*Meiji Pharmaceutical University, Education and Research Center for Pharmacy, Kiyose, Tokyo, Japan*

Background: *Streptomyces coelicolor* A3(2) is a valuable model organism that produces a wealth of secondary metabolites. *S. coelicolor* A3(2) is regulated by numerous essential eukaryotic-type serine/threonine protein kinases (STPKs) in response to environmental cues. However, with the exception of a few STPKs, such as AfsK, the roles of these STPKs are poorly understood.

Objectives: To understand the impact of the STPK PkaE on *S. coelicolor* A3(2) metabolism, we conducted the comparative metabolomic analyses of wild-type *S. coelicolo*r A3(2) with a *pkaE* deletion mutant.

Methods: Metabolites extracted from 50 mg of mycelia from wild-type *S. coelicolor* A3(2) and a *pkaE* deletion mutant grown for 60 hours on GYM agar were identified by capillary electrophoresis with time-of-flight mass spectrometry (CE-TOFMS) (n=5, respectively). Results were analyzed by principal component analysis (PCA), hierarchical clustering analysis (HCA), and Welch's t-test, and mapped onto *S. coelicolor* A3(2)'s metabolic pathway. Choline concentration under the osmotic stress of GYM medium with NaCl was quantified using a Choline/Acetylcholine Quantification Kit (BioVision).

Results: CE-TOFMS identified 304 metabolites (169 cationic and 135 anionic) in the two strains. Bioinformatic analysis revealed that choline and betaine aldehyde were reduced in the *pkaE* deletion mutant compared with that in wild-type (fold change of choline 0.4, $p<0.001$; fold change of betaine aldehyde 0.3, $p<0.001$). Choline and betaine aldehyde are precursor metabolites for glycine betaine, whose relative area increased in the *pkaE* deletion mutant (fold change 1.6, $p<0.001$). Although glycine betaine is an osmoprotectant, choline degradation was not affected by NaCl.

PM107 Phenolic compounds of olive mill wastes exert antibiofilm effects through the target protein WrbA

Cristina Catto'[1], Federico Rossi[1], Gianmarco Mugnai[1], Francesca Cappitelli[1], Fabio Forlani[1], Federica Villa[1]

[1]*Università degli Studi di Milano, Department of Food, Environmental and Nutritional Sciences, Milano, Italy*

Background: Olive oil production generates voluminous waste that has serious environmental and economic impacts. One possible solution is to convert the phenolic content of olive mill wastes (OMW) into non-lethal antibiofilm agents that disarm microorganisms without killing them. According to our previous studies, we speculated that phenolic compounds hinder biofilm formation at non-lethal doses through the modulation of the WrbA enzyme function.

Objectives: This study elucidated the role of WrbA in biofilm genesis, with the final goal of developing an enzyme-based target method for the in-vitro high-throughput screening of phenolic-rich OMW fractions with antibiofilm activities. The work included i) the functional validation of WrbA in biofilm formation and ii) the design of an in-vitro bioassay to measure the antibiofilm activity of phenolic compounds from OMW fractions.

Methods: WrbA functional validation was first carried out by studying the ability of some known antibiofilm phenolic compounds (e.g. salicylic, caffeic, coumaric and cinnamic acids) to modulate WrbA enzyme activity. Then, the WrbA-deprived *E. coli* mutants' ability to form biofilm was tested and compared with that of the isogenic strains. Finally, using OMW fractions with the most promising antibiofilm effects, their effects on target microorganisms and on WrbA enzymatic activity were investigated separately to assess the relation between WrbA and biofilm formation.

Results: Enzyme inhibition assays coupled with biofilm assays of *E. coli* mutants proved that WrbA is a viable molecular target of certain phenolic compounds with antibiofilm activity. The phenolic-rich OMW fractions with WrbA modulatory effect warrant a potential for biofilm prevention.

PM108 Exploration of exopolysaccharide production by cyanothece sp. pcc 7822

Camille van Camp[1], ruddy wattiez[1]

[1]*Mons, Proteomics and Microbiology, Mons, Belgium*

Background: The present research is part of the Algotech project based on the production of high-added value products by Microalgae/Cyanobacteria. In this context, we investigate exopolysaccharide (EPS) production by Cyanobacteria because of their singular properties and applications in cosmetics, medicine or food industries.

Objectives: Examination of culture parameters effects on EPS synthesis and composition of *Cyanothece sp.* PCC 7822 in order to determine future use of the biopolymer at industrial scale.

Methods: This study focuses on the impact of C/N ratio by testing various nitrogen sources ($NaNO_3$, atmospheric nitrogen, NH_4Cl and urea at different concentrations) on bacterium metabolism and EPS production.

Regardless of the nitrogen concentration tested (1 mM, 2.5 mM and 17 mM, and in term of N), $NaNO_3$ induces the best growth of the strain. In parallel, a higher EPS production is observed in presence of $NaNO_3$. Additionally, optical observation of EPS by alcian blue staining highlight variation of exopolysaccharide configuration according to culture condition.

Results: Firsts results demonstrate that C/N ratio variation affects EPS production and configuration implying an impact on EPS composition. Further analyses concerning EPS composition will be of particular interest.

In the frame of a project implicated on microalgal energetics and biomass promotion, the bacterium Cyanothece sp. PCC 7822 reveals its full potential by its EPS composed of carboxylic and especially sulphated group leading to saccharides possessing attractive industrial properties.

PM109 Comparative analysis of biofilm architectures developped by different wild type and null-mutant Acidithiobacillus strains

Mauricio Diaz[1], Diego Serra[2], Regine Hengge[2], Nicolas Guiliani[1]

[1]Universidad de Chile, Faculty of Sciences, Department of Biology, Laboratory of Bacterial Communication, Santiago, Chile
[2]Humboldt-Universität zu Berlin, Institut für Biologie/Mikrobiologie, Berlin, Germany

Background: *Acidithiobacillus* is a predominant and structural species in acidophile communities. *Acidithiobacillus* species have been targeted to decipher the molecular mechanisms involved in biofilm formation by acidophile bacteria due to its role in natural and industrial bioleaching processes. The second messenger cyclic diguanylate has been reported as a molecular player for biofilm formation by *Acidithiobacillus* species. Previously, the structural role of Pel exopolysaccharide in biofilm formation by *At. thiooxidans* has been reported and a *bcsAB* operon involved in cellulose biosynthesis was also described.

Objectives: The main goal was to compare the structural role of different exopolysaccharides in macrobiofilm architecture of *Acidithiobacillus* species.

Methods: Transcriptomic analysis were performed to characterize an *At. thiooxidans* Δ*bcsAB* null-mutant strain obtained by conjugation. Cryomicroscopy and lectin technology were used to compare macrobiofilms architecture developed by *Acidititithiobacillus* species including wild type (*At. caldus*, *At. ferrivorans*, *At. ferridurans*, *At. ferrooxidans*, *At. thiooxidans*) and null-mutants strains (*At. thiooxidans* Δ*pelD or* Δ*bcsAB).*

Results: By using total RNA obtained from planktonic and adhered sulfur-grown cells of *At. thiooxidans*, transcriptomic analysis revealed that *bcsAB* genes are overexpressed in adhered cells. Thioflaving S binding assays indicated that Pel exopolysaccharride and cellulose have different structural role in *At. thiooxidans* macrobiofilm architecture. The viewing of macrobiofilms cross-sections stained with FITC-ConA lectin revealed that the amount of mannose-exopolysaccaride is lower in *At. ferridurans* species than other *Acidithiobacillus* species. Moreover some differences were also observed between two *At. ferrooxidans* strains. However, further studies are still necessary to better understand biofilm architectures of *Acidithiobacillus* sulfur- and iron/sulfur-oxidizing species.

PM110 Analysis of a Novel Plasmid of Halomonas sp. and Their Utilization

Ayaka Tsuji[1], Taku Nishimura[2], Yoshinao Azuma[1]

[1]*Graduate School of Biology-Oriented Science and Technology, Kinokawa, Japan*
[2]*OSAKA GAS Co., Ltd., Energy Technology Laboratories, Osaka, Japan*

Background: *Halomonas* species, halophilic, alkaliphilic and aerobic proteobacteria, are deemed to be useful bacteria to produce various chemicals because of the magnificent ability to accumulate polyhydroxybutyrate (PHB), a bioplastic material, and to secrete pyruvate and 3-hydroxybutyrate(3HB) into the cultures. However, metabolic mechanisms and regulations of these producibilities have not been clarified yet.

Objectives: To establish a platform bacterium to produce a variety of chemicals by fermentation, an environment-friendly technology, we attempted to analyze proteome during PHB production and develop genetic tools using a native plasmid of a *Halomonas* isolate to engineer the metabolism.

Methods: We started to isolate novel, food-born and -related *Halomonas* strains, which showed high accumulation of PHB. Using a plasmid, pHA020_2, identified in an isolate, A020, a shuttle vector for gene transformation and disruption was developed. Highly-expressed and phase-specific genes were selected based on proteome analyses, and activities of the promotors were tested using GFP gene expression.

Results: A fusion vector of pHA020_2 and pUC19 containing chloramphenicol resistant gene successfully transformed *Halomonas* sp. KM-1 and another isolate, *Halomonas* sp. A031. Proteome analysis revealed that Hcp, a type VI secretion system effector, and phasin, polyhydroxyalkanoate-associated protein, were highly accumulated constitutively and in a stationary phase, respectively. Based on reporter assays using GFP, Hcp promoter was activated in any phases and phasin one was weakly done during a stationary phase, while *E. coli* trc promoter was the strongest of all. We are planning to characterize metabolism of *Halomonas* and to make it genetically manipulatable using these genetic tools.

PM111 Protective efficacy of the domains of Erysipelothrix rhusiopathiae surface protective antigen A as vaccine antigens

Hsing-Chieh Wu[1;2], Yi-Ting Lo[1], Chun-Yen Chu[1;2]

[1]*International Program in Animal Vaccine Technology, National Pingtung University of Science and Technology, Pingtung, Taiwan*
[2]*Graduate Institute of Animal Vaccine Technology, College of Veterinary Medicine, National Pingtung University of Science and Technology, Pingtung, Taiwan*

Background: *Erysipelothrix rhusiopathiae*is (*E. rhusiopathiae*is) is a Gram-positive, facultatively intracellular bacterial pathogen that causes erysipelas in swine and a variety of diseases in other animals and humans. It is economically detrimental to pig industries worldwide. Available live-attenuated or bacterin vaccines for swine erysipelas control have thus far shown their effectiveness depends on the susceptibility of the animal to the vaccine. Previous studies indicated that the surface protective antigen A (SpaA) is a common protective antigen produced by various serovars of *E. rhusiopathiae*is.

Objectives: The aim of this study was to evaluate the immunogenic regions of SpaA as potential antigen for subunit vaccines.

Methods: We cloned and expressed four regions of SpaA recombinant protein as full-length (rSpaA-F), N-terminal (rSpaA-N), middle part (rSpaA-M), and C-terminal (rSpaA-C) domains. These recombinant proteins were individually combined with water-in-oil-in-water adjuvant to formulate vaccines. Immune response and protective efficacy of the vaccines were evaluated in mice.

Results: A protection rate of 100% was observed against *E. rhusiopathiae*is challenge in mice vaccinated with the rSpaA-N formulation, and the antibody titer was significantly higher than that of the unimmunized group. We conclude that the N-terminal domain of SpaA may serve as a potent antigen for subunit vaccine development against *E. rhusiopathiaeis*.

PM112 Changes in the antibiotic susceptibility pattern to antibiotics in uncomplicated UTI in outpatients of a tertiary care hospital: Does fosfomycin still stand effective?

Priyam Batra[1], Amit Kumar[2], Stuti Gupta[2], Asif Iqbal[2], Preeti Pushpan[2], Rajendra Kumar[2], Sarman Singh[3]

[1]ALL INDIA INSTITUTE OF MEDICAL SCIENCES, Laboratory Medicine, New Delhi, India
[2]ALL INDIA INSTITUTE OF MEDICAL SCIENCES, Laboratory Medicine, New Delhi,
[3]ALL INDIA INSTITUTE OF MEDICAL SCIENCES, Microbiology, Bhopal, India

Background: Urinary Tract Infections (UTIs) are most common bacterial infections. Patients presenting to OPD with uncomplicated UTI would reflect infection acquired in community. Thus, resistance pattern of organisms isolated would be a reflection of the community scenario. Current study was conducted in a 2300 bedded tertiary care centre of India in patients presenting to OPD with uncomplicated UTI.

Objectives:

a) To study the epidemiology of the pathogens causing UTI in our out patient department.
b) To determine the level of resistance of the pathogens isolated to the various classes of antibiotics available.
c) To determine the level of resistance to oral antibiotics esp fosfomycin
d) To determine the level of carbapenem resistant colistin resistance in the isolates

Methods: Urine samples of all OPD patients presenting with uncomplicated UTI were included. Urine was cultured and isolates obtained were identified using conventional methods. Sensitivity was performed for following antibiotics: ampicillin, amoxycillin/clavulanate, amikacin, nitrofurantoin, norfloxacin, levofloxacin, ciprofloxacin, imipenem, tobramycin, cefepime, fosfomycin and colistin. Results were interpreted as per CLSI guidelines.

Results: Total of 7, 295 isolates of patients with uncomplicated UTI were obtained over 4 years (April 2015- March 2019). 82% of the isolates were gram-negative bacilli (GNBs) with most common isolate being E. coli 3472 (47.6%). Resistance of the GNBs to oral antibiotics in year 2015 and 2018 showed ampicillin (63.8%; 94%), amoxycillin/clavulanate (68%; 93%), ciprofloxacin (51%; 83%), levofloxacin (40.1%; 71.2%), cotrimoxazole (54%; 89.5%), nitrofurantoin (47.2%; 61.2%) and Fosfomycin (0%; 3.9%) respectively. Carbapenem resistance was 2.4% in 2015 and 8.3% in 2018.

PM113 Evaluation of Ulva lactuca as feedstock for fermentation by Clostridia

Eva Maria Ingvadottir[1], Sean Michael Scully[2], Johann Orlygsson[2]

[1]*University of Akureyri, Faculty of Natural Resource Sciences, Iceland*
[2]*University of Akureyri, Faculty of Natural Resource Sciences*

Background: Macroalgae are an abundant and underutilized renewable feedstock that can be exploited for the production of various low- and high-value biomolecules. Examples include *Ulva lactuca,* a green macroalgae species found along Iceland's coasts. It is primarily composed of two polysaccharides: the rhamnose-containing ulvan and the glucose-containing starch. Rhamnose can be microbially fermented to 1,2-propanediol (1,2-PD), a high-value chemical building block which historically has been synthesized from petrochemicals.

Objectives: The fermentation of *Ulva lactuca* hydrolysates and non-pretreated biomass to 1,2-PD using the thermophilic *Clostridia* strain AK1 (isolated from Icelandic hot spring) and *Clostridium beijerinckii* (type strain) for comparison.

Methods: Anaerobic batch-cultures prepared according to a modified Hungate technique. End-products (hydrogen, 1,2-PD, volatiles) analyzed both colorimetrically and using TCD and FID GC. *Ulva lactuca* hydrolysates prepared under neutral and acidic conditions at 10% w/v. Macroalgal constituents measured colorimetrically.

Results: The impact of culture conditions (pH and initial substrate concentration) on rhamnose fermentation was investigated in batch culture. Generally, pH did not influence the production of 1,2-PD from the two *Clostridia* species. *Clostridium beijerinckii* showed substrate inhibition above 40 mM of rhamnose with strain AK1 being sensitive to initial concentrations above 20 mM. When *Clostridium* strain AK1 was cultivated on *Ulva lactuca* hydrolysates and non-pretreated *Ulva lactuca* medium, the maximum production of 0,6 g/L 1,2-PD was obtained from the non-pretreated macroalgae. Thus, *Clostridium* strain AK1 is a potentially promising organism for the consolidated bioprocessing of green macroalgae.

PM114 Fecal Calprotectin level in patients of Ulcerative Colitis attending super speciality hospital in north West India

Arun Kumar Sharma[1], Usha Dutta[2], Saroj Kant Sinha[3], Vishal Sharma[3], Rakesh Kochhar[3]

[1]Post Graduate Institute of Medical Education & Research, Chandigarh, Gastroenterology Department, Chandigarh, India
[2]Post Graduate Institute of Medical Education & Research, Chandigarh, Department of Gastroenterology, Chandigarh, India
[2]Post Graduate Institute of Medical Education & Research, Chandigarh, Department of Gastroenterology, Chandigarh, India

Background: Fecal Calprotectin is a non invasive marker of intestinal inflammation specially in Ulcerative Colitis and Crohn's Disease. Fecal Calprotectin represents majority of neutrophil cytosolic protein present in mucosal lesions of gut. It may be helpful in evaluating disease status in patients of Ulcerative Colitis.

Objectives: To evaluate Fecal Calprotectin levels in stool of ulcerative Colitis patients attending Gastroenterology Department, Nehru Hospital, PGIMER, Chandigarh

Methods: 256(130 M, 126 F)consecutive patients of chronic diarrhea with suspected Ulcerative Colitis(April 2018 to Sept.2018) were included. Clinical features ,diagnostic investigations including colonoscopy were recorded.10-15 grams of stool were collected and tested for quantitative ELISA(cut off $\geq$ 43.2 ugm/gm of stool)

Results: 129 patients(129/256, 50.39%) of mild chronic diarrhoea had mean Fecal Calprotectin of 13.01 ugm/gm of stool.42 out of 265 patients(16.4%) of Ulcerative Colitis under remission during treatment had mean Fecal Calprotectin of 51.84 ugm/gm of stool. The mean Fecal Calprotectin in 85 patients of active Ulcerative Colitis(85/256, 33.2%) was 527.10 ugm/gm of stool. Fecal Calprotectin seems to be a good non invasive marker for knowing disease status in patients of Ulcerative Colitis

PM115 investigating the effect of tobramycin dry powder inhalers on the eradication of pseudomonas aeruginosa biofilms

Reham Aljalamdeh[1], robert price[1], matthew jones[1], albert bolhuis[1]

[1]*university of bath, pharmacy and pharmacology, bath, United Kingdom*

Background: Biofilms are sessile communities of microorganisms embedded within a self-generated extracellular polymeric matrix. Such biofilms are found for instance in adults with cystic fibrosis, with pulmonary infections with the Gram-negative bacterium *Pseudomonas aeruginosa* being particularly common. This infection in CF patients is commonly managed with antibiotic dry powder inhalers, one of which is the aminoglycoside tobramycin. The activity of tobramycin has been well characterized *in vitro*, but current models that have been used are not very representative for lung infections, and better models would provide a significant advantage as these could be used, for instance, to improve the formulation of dry powder inhalers.

Objectives: Our aim is to develop a biofilm model that enables us to test dry powder inhalers. Such as investigating the effect of particle size deposited from a dry powder inhaler antibiotic, tobramycin, on eradication of biofilms.

Methods: we utilized the Next Generator Impactor (NGI), which is a pharmaceutical instrument used to separate particles into size fractions. We used the NGI to separate tobramycin particles into different sizes and tested the influence of these particles on eradication of *P. aeruginosa* biofilms, which were grown using as colony biofilms that closely mimics conditions in the lung where biofilms are grown on a substrate-air interface

Results: Preliminary evidence indicated smaller tobramycin particles are better in eradication of *P. aeruginosa* biofilms as compared to larger particles. Our results may represent a step towards improving the formulation of tobramycin dry powder inhalers to be effective in eradicating *P. aeruginosa* biofilms.

PM116 Functional characterization of a novel class of mobile integrons

Thomas Jové[1], Louise Cerdeira[2], Tamariz Jesus[3], Nilton Lincopan[2;4], Marie-Cécile Ploy[1]

[1]*INSERM, CHU Limoges, RESINFIT, U1092, University of Limoges, Limoges, France*
[2]*Department of Clinical Analysis, Faculty of Pharmaceutical Sciences, University of São Paulo, São Paulo, Brazil*
[3]*University Peruana Cayetano Heredia, Lima, Peru*
[4]*Institute of Biomedical Sciences, University of São Paulo, Department of Microbiology, São Paulo, Brazil*

Background: Integrons are bacterial genetic elements working as "plug-and-play" systems for gene cassettes. Integrons encode an IntI integrase that catalyse the integrations/excisions recombination of gene cassettes into/out of the integron. Moreover, integrons provide a promoter ensuring gene cassette expressions. Five known class of integrons contribute to the dissemination of antibiotic resistance gene cassettes.

Objectives: We identified an unseen integron-like structure in the genome of a multiresistant *Escherichia* coli strain isolated in Peru. We aimed to *in silico* and experimentally investigate its functionality as integron.

Methods: The structure of the integron-like element was analysed using NCBI tools and INTEGRAL. The functionality of its integrase was tested with in *vivo* gene cassette excision and integration assays and the ability to express genes cassettes using *lacZ* transcriptional fusions.

Results: The novel integron-like structure is part of a chromosomal integrative and conjugative element. It encodes a putative IntI integrase with 45-54% identity to other integron integrases and has all conserved catalytic residues. It carries 9 putative gene cassettes, three of them related to antibiotic resistance genes cassettes.

We experimentally showed that **(i)** the encoded integrase is functional for excision of gene cassettes, **(ii)** this integrase can integrate gene cassettes into several classes of integrons and **(iii)** this integron-like element supports the expression of gene cassettes, **(iv)** the expression of the integrase gene is higher than expected and possibly induced by the SOS response.

These evidences suggest that this genetic structure consists in a **6th functional class of integrons for expression dissemination of antibiotic resistance**.

PM117 Investigation of role of horizontal gene transfer in shaping the microbial adaptation to xenobiotics in Pseudomonas

Subarna Thakur[1], Manas Pratim Modak[1]

[1]*University of North Bengal, Department of Bioinformatics, Siliguri, India*

Background: Members of the genus *Pseudomonas* are ubiquitous in nature. They inhibit diverse terrestrial and aquatic habitats including highly polluted environment. Many of them exhibit ability to degrade xenobiotic compounds by their metabolic pathways and been implicated in their role in bioremediation. Genetic exchange by means of horizontal transfer plays a significant role in the evolution and ecological impact of microbe. *In silico* tools can be useful to infer about the horizontal gene transfer (HGT) events.

Objectives: This work aimed at the detection of HGT events in genes involved in metabolism of xenobiotics and their impact in adaptation of various *Pseudomonas* strains to rapidly changing environmental conditions.

Methods: Whole genome of sequence of fifty strains of *Pseudomonas* belonging to various ecological niches was considered for comparative genome analysis. Genes encoding for xenobiotic metabolism were indentified and HGT events were predicted by bioinformatics tools. Sequence composition-based methods were utilized for detecting deviation in codon composition in HGTs. Analysis of evolutionary selection was carried out to determine evolutionary rate of these genes and to detect those under positive selection.

Results: Genomes of bacteria isolated from contaminated surroundings and those involved in host-associations houses large number of xenobiotic genes which are putative HGTs, many of which are located in genomic islands (GIs). Composition based methods highlighted the significance of HGT in the evolution of xenobiotic metabolism across these genomes. Pairwise dN/dS results indicate at the continuous genetic evolution in most of the genomes in order to improve the genetic fitness across stressed environments.

PM118 Comparative microbial pangenomics to explore mobilome dynamics

Bazin Adelme[1], Gautreau Guillaume[1], Médigue Claudine[1], Calteau Alexandra[1], Vallenet David[1]

[1] LABGeM, Génomique Métabolique, CEA, Genoscope, Institut François Jacob, CNRS, Université d'Évry, Université Paris-Saclay, Evry, France

Background: For the last decade, pangenomics has provided new tools for researchers to estimate genomic diversity by partitioning gene content in terms of "core" and "accessory" genome. However, the "core genome" is highly related to the number of genomes included in a study limiting the relevance of comparisons between studies. Moreover, the concept of accessory genome lacks subtlety as it gathers genes with a large range of frequencies. Recently, a new tool named PPanGGOLiN was developed to exploit both gene neighborhood topology and gene frequency to classify gene families using a graph-based approach into a 3-class partitioning of "persistent", "shell" and "cloud" genome.

Objectives: Using this new approach, we are establishing the pangenomes of all microbial species sufficiently represented in databanks. With such a resource, the variable regions of the pangenomes can be detected and split into modules. By studying module conservation between species, we can explore mobilome dynamics in a comparative pangenomics approach.

Methods: Using the graph structure provided by PPanGGOLiN, modules are detected within each pangenome by integrating both co-occurrence and co-localization information. Then we search for conserved modules between species using network alignment methods.

Results: The developed method can be applied on any species with a sufficient number of genomes. The collection of pangenomes with their predicted variable regions and modules will be made available to the research community. This resource may help to associate functional modules that are shared between species with common environmental factors or phenotypic traits.

PM119 In silico and in vivo identification of transposable elements of an opporthunistic pathogen Paracoccus yeei (Alphaproteobacteria)

Dorota Sentkowska[1], Magdalena Szuplewska[2], Cora Chmielowska[3], Albert Roethel[3], Dariusz Bartosik[3]

[1]University of Warsaw, Faculty of Biology, Warszawa, Poland
[2]University of Warsaw, Faculty of Biology, Warszawa,
[1]University of Warsaw, Faculty of Biology, Warszawa, Poland

Background: The genus *Paracoccus* contains hundreds of strains classified into over 50 species, isolated from different environments. Interesting, one species, *Paracoccus yeei*, was found to be associated with opportunistic human infections, which is a unique feature in the entire genus. We speculate that the pathogenic behaviour of these strains may result from acquisition of exogenous genetic information originating from pathogenic bacteria of other species.

Objectives: The main goal of this project was to identify and characterize transposable elements (TEs) of *P. yeei* (by *in silico* and *in vivo* approaches) and to analyze their possible role in dissemination of genetic information related to opportunistic phenotype.

Methods: TEs were predicted *in silico* from genomic sequences of eight clinical and environmental strains of *P. yeei*. Functional TEs were identified using trap plasmids carrying cassettes enabling positive selection of transposition events.

Results: *In silico* analyzes allowed identification of numerous TEs, including: (i) insertion sequences, representing 9 IS families (IS*2*, IS*3*, IS*5*, IS*66*, IS*110*, IS*1182*, IS*30*, IS*256*), (ii) non-composite Tn*3*-family transposons, and (iii) elements encoding non-canonical transposases. This study provided novel and interesting information on: (i) the distribution and genomic localization of TEs in *P. yeei* genomes, (ii) the predicted role of TEs in mobilization for transposition of genomic DNA, and (iii) the dynamics of the process of transposition (identification of elements that transpose with highest frequency).

PM120 Lifestyles are characterized by signature genes in the genus Lactobacillus

Stijn Wittouck[1;2], Vera van Noort[2], Sarah Lebeer[1]

[1]*University of Antwerp, bioscience engineering, Antwerpen, Belgium*
[2]*KULeuven, Centre of Microbial and Plant Genetics, Leuven, Belgium*

Background: In 2017, it was proposed that species of the genus *Lactobacillus* can be classified in three different lifestyles: host-adapted, free-living and nomadic. Furthermore, it was discovered that these lifestyles are largely conserved within subclades of *Lactobacillus*. It is currently unknown whether these lifestyles are reflected by the gene content of the species.

Objectives: We hypothesized that subclades with conserved lifestyle within *Lactobacillus* have relatively large numbers of signatures genes, defined as genes present in each species of the clade but absent in each species outside of the clade. Our objective was to test this hypothesis.

Methods: We gathered one representative genome for each of the approximately 200 species of *Lactobacillus*. We performed gene prediction for all these genomes and clustered the genes into gene families. We then inferred a maximum likelihood tree of the species using the core gene sequences. Finally, we determined the number of signature gene families for each clade in the tree.

Results: We found that the subclades of the *Lactobacillus* phylogeny with conserved lifestyle were characterized by larger numbers of signature genes than most other clades, confirming our hypothesis. This suggests that these signature genes are involved in lifestyle adaptations, making them interesting targets for further research. In addition, our results imply that signature genes could form the basis of new taxonomic criteria that are genome-based but at the same time reflect ecological and metabolic traits.

PM121 Genome Dynamics of Streptomyces clavuligerus

Lis Algora[1], Paul Herron[1]

[1]*University of Strathclyde, Sipbs, Glasgow, United Kingdom*

Background: *Streptomyces clavuligerus* is the producer of clavulanic acid; a β-lactamase inhibitor that is used in combination with amoxicillin. Genome sequencing has established that *S. clavuligerus* contains a linear chromosome and four linear plasmids: pSCL1, pSCL2, pSCL3 and pSCL4. Although pSCL4 carries 20% of the coding sequences, none are thought to be essential with the exception of *tap* and *tpg* that encode terminal proteins necessary for priming of the lagging strand at the telomeres.

Objectives: Study the genome architecture of *S. clavuligerus.* Confirmation of the essentiality of the megaplasmid pSCL4. Study the role of *tap* and *tpg* and the telomeres in *S. clavuligerus*.

Methods: Pulsed-field Gel Electrohpresis is used to analyse the genome architecture of *S. clavuligerus* coupled with Southern hybridisation. Construction of *S. clavuligerus* mutant strains using ϕC31 *att*-site-specific integration and homologous recombination.

Results: The phisical map of the genome of *S. clavuligerus* confirmed the presence of the replicons pSCL2, pSCL3 and pSCL4 with sizes of 150, 450 and 1,800 kilobases respectively. In addition, bioinformatics and physical analyses allowed the identification of similar non-archetypal telomeres in the chromosome and pSCL4 at one end of each replicon. Despite this, the other telomere remains unidentified, which suggests there is a dynamic chromosome-plasmid relationship in *S. clavuligerus.* Furthermore, we introduced a copy of *tap-tpg* onto the chromosome prior to plasmid curing and/or *tap-tpg* deletion. Consequently, targeted genome sequence and further physical analyses will permit us understand the complex dynamic relationship between the megaplasmid and the chromosome in this important industrial microorganism.

PM122 One Aeromonas salmonicida ssp. salmonicida isolate with two unusual plasmids causing antibiotic resistance

Marie-Ange Massicotte[1;2;3], Antony T. Vincent[1;2;3], Anna Schneider[1;3], Valérie E. Paquet[1;2;3], Michel Frenette[3;4], Steve J. Charette[1;2;3]

[1]*Institut de biologie intégrative et des systèmes, Laval University, Quebec City, Canada*
[2]*Centre de recherche de l'Institut universitaire de cardiologie et de pneumologie de Québec, Hôpital Laval, Quebec City, Canada*
[3]*Département de biochimie, de microbiologie et de bio-informatique, Laval University, Quebec City, Canada*
[4]*Groupe de Recherche en Écologie Buccale, Laval University, Quebec City, Canada*

Background: Furunculosis is a disease affecting mainly farmed fish in the salmonids family characterized by high mortality rates. Caused by the aquatic bacterium *Aeromonas salmonicida* ssp. *salmonicida* (*A.sal.*), this disease has major economic impacts on aquaculture operations worldwide. The effectiveness of antibiotherapy to treat furunculosis is becoming limited due to the emergence of resistant and even multidrug-resistant *A.sal.* strains through the acquisition of resistance genes, usually carried by plasmids.

Objectives: We annually carry out genomic analyses of new *A.sal.* isolates to identify potentially new antibiotic resistant plasmids.

Methods: In this way, by using PacBio sequencing and confirmed by appropriate PCR genotyping, we discovered and characterized two novel unusual plasmids recovered from the same isolate and harboring antibiotic resistance genes.

Results: Those plasmids, named pAsa5-3432 and pRAS3-3432, are particularly interesting since they are both arising out of rare genetic events. The recombination between two plasmids led to the formation of pAsa5-3432. This plasmid now bears both an essential virulence factor (from pAsa5) and multiple antibiotic resistance genes (from pAsa8), a feature never previously reported in *A.sal.*. Without PacBio sequencing, this plasmid fusion would not be detected. On its side, plasmid pRAS3-3432 carries a new mobile element that has only been identified in the pig pathogen *Chlamydia suis*. This study brings further evidence that *A.sal.* owns a rich plasmid repertoire that contributes to the spread of antibiotic resistance genes into the aquatic environment and suggests that plasmid fusion may exist in other isolates which can be revealed only by long-read sequencing technologies.

PM123 A new class of spliceosomal twin intron (stwintron) in Aspergillus nidulans and its role in gene expression through alternative splicing

Napsugár Kavalecz[1], Norbert Ág[1], Levente Karaffa[1], Michel Flipphi[1], Erzsébet Fekete[1]

[1]*University of Debrecen, Department of Biochemical Engineering, Debrecen, Hungary*

Background: Stwintrons are unconventional intervening sequences where a standard "internal" intron interrupts one of the canonical splicing motifs of an "external" intron. This lariat branch point motif-interrupted stwintron ([L4,5]: 5'-GCUA|AU) occurs in the gene coding for a variant form of heat shock protein 12 in *Aspergillus nidulans*, but lacks from the orthologue gene in related species (*A. sydowii*, *A. calidoustus*).

Objectives: Excision of the external intron removes the only possible translational start codon from the transcript, implying that the RNA requires to retain the external intron to deliver a protein – a form of alternative splicing known as "intron retention".

Methods: To confirm the existence of the stwintron, reverse transcription PCR (RT-PCR) reactions were used. To study the expression of the gene and thus alternative splicing, we used osmotic shock.

Results: We show that the steady state levels of the RNA species from which only the internal intron is excised, increase considerably when *A. nidulans* biomass is transferred from a minimal medium without extra salt to one including 1 M NaCl, and decreases back to its initial levels when the biomass is subsequently transferred back to a medium without added salt. In an example of genetic flexibility provided by intron sequences, the chance formation of the external intron has offered the fungus an opportunity to develop extra means of regulation of the variant HSP12 protein in extant in *A. sydowii* and *A. calidoustus*.

This work was supported by the ÚNKP-18-3 New National Excellence Program of the Ministry of Human Capacities.

PM124 Genomic analysis of reconstruction of the catabolic potential and halotolerance of Acinetobacter sp. DD78

Constanza Macaya[1], Valentina Mendez[1], Roberto E. Duran[1], Francisco Salva-serra[2;3], Constanza Cardenas[4], Edward Moore[2;3], Michael Seeger[1]

[1]Universidad Tecnica Federico Santa Maria , Chemistry Department, Valparaiso, Chile
[2]Sahlgrenska University Hospital, Department of Clinical Microbiology, Culture Collection University of Gothenburg, Gothenburg, Sweden
[3]Centre for Antibiotic Resistance Research (CARe), University of Gothenburg, Gothenburg, Sweden
[4]Pontificia Universidad Catolica Valparaiso, Nucleo Biotecnologia Curauma, Valparaiso, Chile

Background: *Acinetobacter* sp. DD78 is an aerobic hydrocarbonoclastic bacterium that was isolated from Aconcagua river mouth. Strain DD78 possess a high emulsification capability and has been used in bioremediation of non-saline soils. Soil salinity is a limiting factor in bioremediation processes, causing an unbalanced osmotic stress across the bacterial cell wall. Molecular adaptation involves synthesis and uptake of compatible solutes and synthesis of transporters.

Objectives: The aims are to identify and characterize genes involved in hydrocarbon catabolic pathways, adaptation to halotelerance, and the alasan anabolic pathway in the *Acinetobacter* sp. DD78 genome.

Methods: Genome sequencing was performed using PacBio RSII platform. Reads were assembled with HGAP, v3.0. Annotation was performed using Prokka. Genes were identified by BLAST, using Uniprot-KB/Swiss-Prot database.

Results: DD78 genome (3.25 Mbp) harbors 3,102 coding sequences distributed in four replicons: one chromosome (3 Mb) and three plasmids (90, 81 and 70 kb). Overall G+C content of the genome is 41%. 16S rRNA gene sequence analysis revealed that strain DD78 is closely related to *A. radioresistens* (99%). Genes associated with alkanes catabolic pathways were identified. Strain DD78 harbors the genes of a several catabolic pathways of aromatic compounds (*e.g.*, benzoate and anthranilate). Strain DD78 was able to grow up to 4% w/v NaCl. Betaine biosynthetic genes (*betICBA*), proline/betaine and glycine/betaine transporters involved in halotolerance were identified. In addition, alasan anabolic genes (*alnA*, *alnB*, and *alnC*) and their genes products were characterized. This study suggests that *Acinetobacter* sp. DD78 will be useful for bioremediation of hydrocarbon-polluted saline soils

PM125 Comparative genomics studies revealed the effect of artificial selection on the genome of Ustilago esculenta

Zihong Ye[1], Yafen Zhang[1], Haifeng Cui[1], Wenqiang Xia[1], Xiaopin Yu[1], Yang Song[1]

[1]*China Jiliang University*

Background: *Ustilago esculenta*, a smut fungus, infects *Zizania latifolia* to form galls named *Jiaobai* in China. Their differentiation induced *Z. latifolia* to form edible white *Jiaobai* (MT strains) and harmful grey *Jiaobai* (T strains). Studies showed a growth and infection defect in MT strains, which displayed an absolutely stable endophytic life in the host without an infection cycle, while T strains are phytopathogens.

Objectives: To study the long-standing artificial selection that maximizes the occurrence of favorable white *Jiaobai*, and its effects on maintaining the plant-fungi interaction and modulating the fungus evolving from pathogen to entophyte.

Methods: The 20.2 Mb *U. esculenta* draft genome of 6,654 predicted genes including mating, primary metabolism, secreted proteins were identified through whole genome sequencing analysis.

Results: The genome sharing a high similarity to related Smut fungi, but lost some surface sensors, important virulence factors and host range related effectors, especially in the MT strains. Besides, some immune response genes to PAMPs and PRRs are mutated in *Z. latifolia*. These attenuated pathogenicity-resistance relationships make for the economic endophytic life of *U. esculenta*. Additionally, *U. esculenta* prefers RNA silencing not RIP in defense and has more introns per gene, indicating relatively slow evolution rate. The fungus also lacks some genes in amino acid biosynthesis pathway which were filled by up-regulated host genes, and developed distinct amino acid response mechanism to balance the infection-resistance interaction. These findings can not only contribute to more comprehensive insights into the molecular mechanism underlying artificial selection but also into smut fungi-host interactions.

PM126 Genome reconstruction and comparative analysis of five new Mycobacterium avium subsp. paratuberculosis isolates

Jaewon Lim[1], Hong-Tae Park[2], Gyumin Lee[1], Donghyuk Kim[1], Han Sang Yoo[2]

[1]Ulsan National Institute of Science and Technology, School of Energy and Chemical Engineering, Ulsan, Korea, Rep. of South
[2]College of Veterinary Medicine, Seoul National University, Department of Infectious Diseases, Seoul, Korea, Rep. of South

Background: *Mycobacterium avium* subsp. *paratuberculosis* (MAP) causes Johne's disease (paratuberculosis) in cattle, which is a chronic debilitating disease. Scrutinizing genomes of MAP isolates is pivotal to understand how MAP infects their hosts and which genes are involved in that process. In this study, we sequenced and analyzed five newly isolated MAP strains to determine the genomic features of reconstructed genomes.

Objectives: Analysis of the genomes of MAP isolates and establish their genomic features with comparative genomic analysis

Methods: Five MAP strains were isolated in the three different regions of Korea. For strain selection, Herrold's egg yolk medium with mycobactic J was used for culture. Genome sequencing was performed by PacBio RSII sequencing platform and the sequencing reads were assembled with HGAP. National Center for Biotechnology Information (NCBI) Prokaryotic Genome Annotation Pipeline (PGAP) was used to annotate the genomes. In order to conduct comparative genomic analysis, Bacterial Pan Genome Analysis (BPGA) tool and Clusters of Orthologous Groups (COGs) database were applied.

Results: The genome size and the gene number of five new MAP isolates were 4,836,474 bp (GC content 69.3%) and 4,604 in average. The following comparative genomic analysis was performed in two hierarchical groups; A: five MAP in this study and B: 39 MAP stored on NCBI. In this result, function and distribution of each gene were figured out. This work was supported by the Strategic Initiative for Microbiomes in Agriculture and Food, MAFRA (No. IPET918020-4), the BK21 PLUS and RIVS, SNU, Korea.

PM127 Evolutionary dynamics of all integron resistance cassettes

Alberto Hipólito[1], Filipa Trigo de Roza[1], Thomas Jové[2], Alvaro San Millan[3], José Antonio Escudero[1]

[1]*Universidad Complutense de Madrid, Sanidad Animal, Madrid, Spain*
[2]*Université de Limoges, Faculté de médecine, Limoges, France*
[3]*Ramón y Cajal Health Research Institute, Microbiology, Madrid, Spain*

Background: Antibiotic resistance is postulated as one of the greatest health problems in the 21st century. Integrons are genetic platforms able to capture and stockpile new genes embedded in small mobile elements called cassettes, forming cassette arrays. They act as vehicles for dozens of resistance genes, and are likely the main genetic element causing multidrug resistance in Gram negatives. Despite their importance, little is known about the adaptive value and fitness cost of resistance cassettes.

Objectives: We seek to identify all different integron resistance cassettes described to date, and to study their adaptive value (resistance level), fitness cost and transcriptional interference with downstream cassettes.

Methods: Integrall database has been screened for resistance genes using a cut off value of 95% in sequence identity.

Results: We have retrieved 177 different cassettes conferring resistance to 12 families of antibiotics. Of these, 160 were successfully synthesized *in vitro*. We have developed a vector (pMBA) in which cassettes are cloned under the control of a PcW promoter-mimicking their native genetic environment- and followed by a GFP. pMBA will allow us to establish the resistance levels of each gene, their fitness cost and their impact on transcription/translation of downstream genes (GFP reporter). These are key features in the evolutionary dynamics of integron cassettes that have not been studied systematically. Our data will help understand the adaptive value of resistance integrons, which is crucial to limit their spread.

PM128 Role of a Novel Two-Component System, BSF29-3_0788 and BSF29-3_0791, in Bacillus subtilis F29-3

Yu-Zhuang Lin[1], Hung-Yu Shu[2], Guang-Huey Lin[3]

[1]*Tzu Chi University, Department of Life Science, Hualien, Taiwan*
[2]*Chang Jung Christian University, Department of Bioscience Technology, Tinan, Taiwan*
[3]*Tzu Chi University, Master Program in Microbiology and Immunology, Hualien , Taiwan*

Background: Two-component systems (TCSs) play an important role for bacteria to adjust to environmental conditions. Polyketide is a large group of secondary metabolisms which have bioactive property and commercial value. In previous study, Bacillus subtilis F29-3 is an antagonistic bacterium which can against a wide range of fungal species. Recently, A pair of genes encoding the novel bacterial two-component regulatory system, BSF29-3_0788/0791, was found next to an uncharacterized polyketide synthesis gene cluster (pksF locus) of B. subtilis F29-3 through whole gene analysis.

Objectives: Investigate the role of a novel two-component system, BSF29-3_0788 and BSF29-3_0791, in Bacillus subtilis F29-3.

Methods: First, we construct BSF29-3_0788, BSF29-3_0791 and pksF mutant strain by markerless deletion and transposon mutagenesis. Then, we investigate the role of BSF29-3_0788/0791 in B. subtilis F29-3 via compare phenotype of wild type and mutant strain with polyketide quntification, disk diffusion, growth curve assay and morphology survey.

Results: Outcome indicated that the BSF29-3_0791 mutant and *pks*F mutant strain showed a significant decrease in the polyketide production and increase of organic hydroperoxide resistance ability when compared to the wild-type, but there is no different in morphology, growth curve and non-hydroperoxide resistance ability between wild type and the mutant strain. We can conclude that the novel TCS, BSF29-3_0788/0791 may regulate the uncharacterized polyketide production and organic hydroperoxide tolerance in *B. subtilis* F29-3. Through this study, we can understand how the novel TCS regulate polyketide production and other physiology of *B. subtilis* F29-3, and it will provide valuable references for industrial fermentation in future.

PM129 Deciphering genomic markers to predict plant pathogenicity in Erwinia genus

Felipe Lira[1], Gilles Hunault[2], Martial Briand[1], Perrine Portier[1], Marie-Agnès Jacques[1], Claudine Landès[1], Marion Fischer-Le Saux[1]

[1]IRHS, Agrocampus-Ouest, INRA, Université d'Angers, SFR 4207 QuaSaV, 49071, Beaucouzé, France
[2]Université d'Angers, Laboratoire Hémodynamique Interaction Fibrose et Invasivité Tumorale Hépatique, HIFIH, UPRES 3859, IFR 132, Faculté des Sciences, Angers, France

Background: The genus *Erwinia* is composed of species which can be classified as plant-pathogenic and non-plant-pathogenic. However, the actual methods to phenotype pathogenicity are very time-consuming and dependent on microbiological techniques. Comparative genomics permits to check *in silico* the presence of genomics elements which can be used to distinguish pathogens from commensals.

Objectives: The aim of this study is to perform a comprehensive comparative genomics analysis of closely related strains and species from the genus *Erwinia* to describe their genetic variability and to identify genomic elements capable to serve as markers to distinguish pathogenic from non-pathogenic individuals.

Methods: Fifty-nine genomes from genus *Erwinia* were analyzed using a combination of strategies to capture genomic markers. The PrediPath dataset was customized using 36.561 proteins from public repositories related to virulence and to antimicrobial and heavy metal resistance. A second dataset of 219.499 clusters of genes related to secondary metabolite compounds was also used. A workflow was designed to search presence/absence of elements from these datasets in each genome. Lastly, a not *a priori* approach was applied to detect specific shared *k-mers* among plant-pathogenic erwinias.

Results: The three strategies allowed us to decipher exclusive features for each group analyzed. Statistical analysis of the results obtained with the PrediPath dataset indicated *Hrp* cluster as critical predictor of pathogenicity in genus *Erwinia.* 1.711 secondary metabolite clusters distributed into 29 categories were identified in the 59 genomes and demonstrated species-specific occurence. The *k-mer* approach recognized a limited number of exclusive short sequences for each group analyzed.

PM130 MicroScope: an integrated platform for the annotation and exploration of microbial gene functions through genomic and metabolic comparative analysis

David Vallenet[1], Alexandra Calteau[1], Mathieu Dubois[1], Mylène Beuvin[1], Laura Burlot[1], Xavier Bussell[1], Stéphanie Fouteau[1], Guillaume Gautreau[1], Aurélie Lajus[1], Jordan Langlois[1], Rémi Planel[1], David Roche[1], Johan Rollin[1], Zoé Rouy[1], Claudine Médigue[1]

[1]LABGeM, Génomique Métabolique, CEA, Genoscope, Institut François Jacob, CNRS, Université d'Évry, Université Paris-Saclay, Evry, France

Background: Large-scale genome sequencing and the increasingly massive use of high-throughput approaches produce a vast amount of new information that completely transforms our understanding of thousands of microbial species. However, despite the development of powerful bioinformatics approaches, full interpretation of the content of these genomes remains a difficult task.

Objectives: To address this challenge, we develop the MicroScope platform, which is an integrated Web platform for management, annotation, comparative analysis and visualization of microbial genomes (https://www.genoscope.cns.fr/agc/microscope).

Methods: Published for the first time in 2006, the platform has been under continuous development within the LABGeM group at Genoscope and provides analysis for complete and ongoing genome projects together with metabolic network reconstruction and post-genomic experiments allowing users to improve the understanding of gene functions.

We will present an overview of the MicroScope analysis pipelines and illustrate the use of several new functionalities in the context of data discovery and expert annotation, which concern:

- automatic annotation by UniFire software based on UniRule system,
- annotation of virulence and antimicrobial resistance genes,
- comparative genomics with synteny computations and pan-genome analyses,
- prediction of regions of genomic plasticity and their characterization, like, secretion systems, integrons and secondary metabolite biosynthesis gene clusters,
- and metabolic network reconstruction.

Results: To date, MicroScope contains data for >10,000 microbial genomes, part of which are manually curated and maintained by microbiologists (>4,000 personal accounts in January 2019). The platform enables collaborative work in a rich comparative genomic context and improves community-based curation efforts.

PM131 Novel natural products biosynthetic potential of the class Ktedonobacteria

Yu Zheng[1], Ayana Saitou[2], Chiung-Mei Wang[1], Atsushi Toyoda[3], Yohei Minakuchi[3], Yasuteru Sakai [1], Keietsu Abe[1], Akira Yokota[1], Shuhei Yabe[1]

[1]*Tohoku University, Graduate School of Agricultural Sciences, Sendai, Japan*
[2]*Tohoku University, Faculty of Agriculture, Sendai, Japan*
[3]*National Institute of Genetics, Mishima, Japan*

Background: The prevalence of antimicrobial resistance and decrease of novel antibiotic discovery promote us to unearth potentially novel natural products biosynthetic microbial resources. *Ktedonobacteria*, a class of deeply branched bacterial lineage in phylum *Chloroflexi*, are ubiquitous in terrestrial environments and share similarities with the traditional microbial resources *Actinobacteria* in relatively large genome size and complex life cycle. These characteristics indicate *Ktedonobacteria* a potential active producer for bioactive compounds.

Objectives: In this study, we conducted genome mining of nine *Ktedonobacteria* strains to evaluate their poteintial to produce novel bioactive compounds. Antimicrobial activity against bacterial and fungal pathogens were also screened.

Methods: antiSMASH 3.0 was used to identify putative secondary metabolites biosynthetic gene clusters (SMBGCs). Functional and evolutionary phylogenetic analysis of the nonribosomal peptide synthetases (NRPS) Condensation (C) domain and polyketide synthases (PKS) Keto-Synthase (KS) domain were analysised by NCBI Protein-Protein Blast and NaPDoS. Antimicrobial activity were assessed by paper disc and MIC method.

Results: A total of 104 putative SMBGCs were identifed in nine *Ktedonobacteria* genomes with high novelty and diversity. Functional and evolutionary phylogenetic analysis of the C domains and KS domains further supported *Ktedonobacteria* may produce structurally different compounds with novel mechanism. Furthermore, bioactive screening of representative *Ktedonobacteria* strains resulted in broad antibacterial and antifungal activities. Herein, in this study, we propose the ancient, ubiquitous, and spore-forming *Ktedonobacteria* a talented and promising microbial resource for natural products discovery.

PM132 Genetic analysis of new Listeria monocytogenes genomes for antimicrobial resistance

Ogueri Nwaiwu[1]

[1]*University of Nottingham, Nottingham, United Kingdom*

Background: The organism *Listeria monocytogenes* is a foodborne pathogen that is known to be a concern for many at-risk groups. Presently, antimicrobial resistance (AMR) is a big concern and there is a consensus that new isolates should be screened for AMR, hence a genotypic screening was performed on new strains of the organism.

Objectives: The objective of the study was to compare new tropical *L. monocytogenes* genomes with other new genomes and establish the nature of AMR present.

Methods: Strains of the organism were isolated from leafy vegetables using standard methods after which their molecular serotypes were determined. Whole genome sequencing was carried out and then genotypic analysis of the contigs present in the genome assemblies of the new strains was performed to determine if they possess AMR.

Results: Three AMR gene families were detected and they included phosphatidylglycerol lysyltransferase (*mprF*) which acts on peptides via antibiotic alteration, fosfomycin thioltransferase (*FosX*) that acts on fosfomycin through inactivation and a major antibiotic efflux pump gene (*norB*) that facilitates fluoroquinolone antibiotic resistance. The resistant genes percentage identity of the matching region found in the new isolates varied with some well-characterized *L. monocytogenes* strains. Resistance genes found in food-borne pathogenic bacteria are problematic and it would be beneficial to carry out more studies to confirm the actual differencies with reference strains and ascertain the level of phenotypic resistance shown by the new isolates.

PM133 Are horses a reservoir for high level gentamicin resistance? Emergence in Streptococcus dysgalactiae subsp. equisimilis in France

Tony Rochegue[1], Philippe Glaser[2], Nicolas Cabanel[2], Estelle Saras[1], Marisa Haenni[1], Jean-Yves Madec[1], Agnese Lupo[1]

[1]*ANSES, Lyon, France*
[2]*Institu Pasteur, Unité Ecologie et Evolution de la Résistance aux Antibiotiques, Paris, France*

Background: High-level gentamicin resistance (HLGR) is of concern in streptococci as it abolishes the synergy with beta-lactams and impedes rapid killing of these pathogens. So far, HLGR streptococci have been reported exclusively from humans.

Objectives: Through the Resapath French veterinary network, in 2017, three *Streptococcus dysgalactiae* subsp. *equisimilis* demonstrating HLGR (E-test >1024 mg/L) were found in unrelated horses, with reproductive disorders. This study aimed at investigating the genetic mechanisms underlying the HLGR.

Methods: The isolates were sequenced by Illumina MiSeq. Assembly of the reads was performed using Spades 3.9. The sequence type, presence of SNPs and resistance genes was determined by MLST 2.0, CSI Phylogeny 1.4 and ResFinder, respectively. Genomic islands were analysed by BLASTN.

Results: The *aph*(2')-*Ib* and *aac*(6')-*Im* genes, known to confer HLGR, were found in all isolates on a 6.7kb genomic island, also containing genes related to conjugative transfer (*traA*, *traD*, *tnpW*), in turn inserted in an *ICE*-like element harbouring an *lsa*(C) gene. The isolates were clonal and belonged to ST196. They were resistant also to tetracycline and harboured the *tet*(M) gene carried by Tn*916*. This is the first report of HLGR in streptococci from animals. The co-occurrence of *aph*(2')-*Ib* and *aac*(6')-*Im* genes have been reported so far in one *Enteroccus faecium* and one *Escherichia coli* from humans in US. Our study, which provides for the first time the genetic environment of these genes, thus suggests the potential for a wide dissemination. The emergence of HLGR in horses puts humans at risk of contagion and warrants monitoring.

PM134 Characterization of a large blaVIM-2-carrying plasmid in Pseudomonas portuensis sp. nov., a novel species within the Pseudomonas putida phylogenetic group

João Francisco Tomaz Santos Botelho[1], Joana Rocha[1], Cédric Lood[2;3], Sally Partridge[4], João A. Carriço[5], Teresa Goncalves Ribeiro[1], Vera van Noort[2;6], Rob Lavigne[3], Filipa Grosso[1], Luisa Maria Vieira Peixe[1]

[1]UCIBIO/REQUIMTE, FFUP, Microbiology, Porto, Portugal
[2]Centre of Microbial and Plant Genetics, KU Leuven, Department of Microbial and Molecular Systems, Leuven, Belgium
[3]Laboratory of Gene Technology, KU Leuven, Department of Biosystems, Leuven, Belgium
[4]Centre for Microbiology and Infectious Diseases, The Westmead Institute for Medical Research, The University of Sydney, Westmead Hospital, Sydney, Australia
[5]Instituto de Microbiologia, Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Lisboa, Portugal
[6]Institute of Biology, Leiden University, Leiden, Belgium

Pseudomonas putida group represents a diverse cluster of opportunistic pathogens and environmental species. Previous studies have addressed the phylogeny in pseudomonads and have urged the need for genome sequencing type strains and correct species assignment to provide more robust phylogenomics.

Here, we conducted genotypic and phenotypic analyses to define the taxonomy and the pool of antimicrobial resistance genes present on a *Pseudomonas* spp. strain recovered from a Portuguese patient with pneumonia.

MinION and Illumina reads were combined to fully assemble the genome. Taxonomic classification was based on multilocus sequence analysis (MLSA), average nucleotide identity (ANI), genome-to-genome distance calculation (GGDC) and biochemical tests. Prokka and Galileo AMR were used for annotation and to explore the genetic context of antimicrobial resistance genes, respectively.

FFUP_PS_41^T strain was Gram-stain-negative, rod-shaped, motile and growth at 4-37ºC. The GC content of FFUP_PS_41^T strain was 62.2%. The MLSA, ANI and GGDC values below the species cutoff confirmed that the strain belongs to a novel species within the *P. putida* phylogenetic group. The FFUP_PS_41^T strain carries a In103 class 1 integron with a |*aacA7*|*bla*$_{VIM-2}$|*aacA4*| cassette array encoding resistance to beta-lactams (including carbapenems) and aminoglycosides on a 498,516-bp megaplasmid. This untypable plasmid (named pJBCL41) harbors a full set of genes for self-transmission and genes predicted to be responsible for plasmid replication, partitioning and maintenance, antibiotic and heavy metal resistance. Biochemical characteristics further differentiated the strain from other species of the genus *Pseudomonas,* for which the name *Pseudomonas portuensis* sp. nov. (FFUP_PS_41^T) is proposed.

PM135 The infectivity decay in Ustilago esculenta mediated by transposons

Wenqiang Xia[1], Zihong Ye[1], Yafen Zhang[1], Haifeng Cui[1]

[1]*China Jiliang University, College of life sciences*

Background: *Ustilago esculenta*, a smut fungi, can infect *Zizania latifolia* and induce plant tomours at the base of host stem. Previous studies have showed that the infectivity decay and prolonged incubation period lead to the formation of plant tomours with few teliospore inside, which is edible and called *Jiaobai* in China. Different strains were isolated from tomours with few (MT type) or abundant (T type) telispore.

Objectives: Transposons are highly variable cis-element on genome, which can generate genomic novelty by chromosome rearrangements and gene expression regulations. We tried to study the roles of transposons in the differenation of MT and T type *U. esculenta*.

Methods: Firstly, transposon sequences are predicted by repeat sequence and recent transposition events on *U. esculenta* genome. Then 36 strains, including MT and T type, are isolated from various regions of China and prepared for Illumina resequencing. Insert positions of transposons are analysed and compared between T and MT type.

Results: Transposons are found more frequently near the virulence gene clusters that are consist of tandem genes encoding secreted proteins, indicating that transposons may be involved in the evolution of it. The disperal of virulence gene clusters found in U. esculenta may result from the high density of transposons. The inserting sites of transposons vary significantly in T type strains, however, keep much more stable in MT type strains. Several different inserting site identified between T and MT type is coupled with differently expressed virulence genes near it, which may be responsible for the infectivity decay.

PM136 New strains of cyanobacteria Trichormus isolated in temperate and Antarctic regions are members of clearly designated subgroup of Trichormus variabilis strains

Lidia Mikheeva[1], Elena Karbysheva[1], Oxana Zhenavchuk[1]

[1]*Moscow State University, Department of Genetics*

Background: Recently taxonomically revised *Trichormus* clade appears to be morphologically and phylogenetically heterogeneous. Our focus is on clearly defined subgroup of *Trichormus* strains closely related to model cyanobacterium *T. variabilis* ATCC 29413. Our collection of such strains, which until recently consisted mainly of subtropical isolates, was supplemented with isolates from the Antarctica and the Moscow region.

Objectives: Our aim was the isolation of new *Trichormus* strains and their genetic analysis including genome sequencing.

Methods: We have used standard methods of isolation of nitrogen fixing cyanobacteria from biofilms on particles of hypolithic soil-like system (Larsemann Hills oasis, East Antarctica). The second set of isolates were derived from close association with mosses plants *Marchantia polimorpha* and *Dicranum scoparium* in Moscow region. Microscopic study of cell morphology, PCR fingerprinting and NGS sequencing were used for comparative analysis of three new isolates, two subtropical *Trichormus* simbionts of *Azolla* and two Siberian strains (from Limnological Institute, Irkutsk).

Results: Sequencing data have revealed the high degree of genetic homology between all *T. variabilis* isolates, namely the presence of unique genomic elements of reference genome *Trichormus variabilis* ATCC 29413 such as plasmid D and small linear chomosome, minimal degree of single-nucleotide polymorphism and the rare intragenomic rearrangements. This is very unusual phenomenon for independent strains originating from so extremely different geographical and climatic zones. It is obvious that complex, stable and highly optimized genome provides the wide distribution and high adaptive capacity of *Trichormus variabilis* strains.
This work has been supported by the RFBR project 17-04-01098

PM137 Tad-like pili system in Chloroflexi phylum

Vasil Gaisin[1], Romain Kooger[2], Martin Pilhofer[2], Vladimir Gorlenko[3]

[1]Research Center of Biotechnology of the Russian Academy of Sciences, Institute of Bioengineering, Moscow, Russian Federation
[2]ETH Zürich, Institute of Molecular Biology & Biophysics, Zürich, Swaziland
[3]Research Center of Biotechnology of the Russian Academy of Sciences, Winogradsky Institute of Microbiology, Moscow, Russian Federation

Background: The mechanisms underlying motility in phototrophic Chloroflexi bacteria are virtually unknown. These multicellular filamentous bacteria are capable of cellular aggregation, surface adhesion and surface-dependent motility. Pili have been shown to mediate twitching motility in very diverse bacterial lineages. Recently, pili were detected on the cell surface of two thermophilic Chloroflexi bacteria: Chloroflexus aggregans and C. islandicus. These findings hint at a central role of pili in the surface-dependent motility in Chloroflexi bacteria.

Objectives: Our goal was to study the evolution and structure of pilus-like organelles in Chloroflexi bacteria.

Methods: We applied two approaches:

(i) Comparative analysis of publicly available Chloroflexi genomes for the prediction of genetic determinants of pilus biogenesis and for the understanding of their evolution.

(ii) Ultrastructural analysis based on cryo-electron tomography (cryo-ET) of plunge-frozen cells of motile C. aggregans DSM-9485 and Roseiflexus castenholzii DSM-13941 species.

Results: Currently, gliding motility is described as the prevalent mechanism for motility in phototrophic Chloroflexi. However, the individual multicellular filaments have twitching-like motility as we observed using light microscopy. Comparative genomic analysis showed that the genomes of most phylogenetic lineages within the Chloroflexi phylum contain genes of the Tad-like pili system. CryoET on C. aggegans and R. castenholzii revealed pili anchored near the intercellular junction between two cells of a multicellular filament. Their localization is consistent with the pili-based motility model described for multicellular filamentous cyanobacteria. Based on our results we suggest that Tad pili could be involved in surface-depend motility and adhesion of Chloroflexi bacteria.

PM138 Role for Spliceosomal Twin Introns in Two Models of Alternative Splicing

Napsugár Kavalecz[1], Norbert Ág[1], Levente Karaffa[1], Michel Flipphi[1], Erzsébet Fekete[1]

[1]*University of Debrecen, Department of Biochemical Engineering, Debrecen, Hungary*

Background: In the primary transcript of nuclear genes, coding "exons" usually alternate with non-coding "introns". The latter are precisely excised by the U2 spliceosome to create the ORF that translates into the correct peptide. Spliceosomal twin introns ("stwintrons") are complex intervening sequences where an "internal" U2 intron interrupts one of the canonical splicing motifs of an "external" U2 intron (viz. 5'-donor; 3'-acceptor; motif around branch-point A) and consequently, are removed by two consecutive splicing reactions.

Objectives: Originally, alternative splicing was presented as a means to increase protein diversity but more often it yields "dysfunctional" RNAs (not encoding the correct peptide), which are rapidly degradated by nonsense-mediated mRNA decay. We investigated functional relations between *bona fide* stwintrons, and extant exon skipping and intron retention events.

Methods: Public DNA databases, simple bio-informatics, RT-PCR.

Results: A donor-disrupted stwintron in a ubiquitous gene occurs broadly in the Pezizomycotina subphylum. The stwintron is crucially involved in "skipping" the exon behind it in certain species, like *Aspergillus niger*, by using alternative 3'-splice sites for its internal intron. A branch-point motif-interrupted stwintron was found in *Aspergillus nidulans*. Orthologue genes in related species specify a standard intron at the same position as the internal intron of the *A. nidulans* stwintron. Excision of the new external intron removes the AUG, implying that it must be retained to deliver a protein. Research was supported by the EU and co-financed by the European Regional Development Fund under the project GINOP-2.3.2-15-2016-00008. EF is supported by the "New National Excellence Program" (ÚNKP-18-4-DE-3).

PM139 Plasmid Replication Associated Single-Strand Specific Methyltransferases

Alexey Fomenkov[1], Iain A. Murray[1], Zhiyi Sun[2], Brian P Anton[1], Tamas Vincze[2], Yoshiharu Yamaich[3], Richards J. Roberts[1]

[1]*New England Biolabs, Genome biology, Ipswich, United States*
[2]*New England Biolabs, Bioinformatic, Ipswich, United States*
[3]*CNRS Gif-sur-Yvette, Gif-sur-Yvette , France*

Background: : About 48% of organisms harbor active Type II MTases with no apparent cognate restriction enzyme. These 'orphan' MTases exhibit patterns of incomplete methylation that distinguish them from RM system MTases, and indicate potential functions in regulation of gene expression and DNA replication in diverse bacterial and archaeal phyla.

Objectives: The genome and methylome analysis of total DNA from two pathogenic strains, *Burkholderia cenocepacia* J2315 and *E.coli* O104:H4 has revealed the presence of two unusual MTases not previously characterized. Both were plasmid-encoded by ORFs in pBCA072 for *B. cenocepacia* J2315 and pESBL for *E. coli* O104:H4. Moreover, BLAST searching identified more than 1000 potential DNA MTase homologues in GenBank, the vast majority of which are clearly plasmid-associated.

Methods: Next Generation SMRT sequencing technology allows not only the sequence and assembly of a variety of genomes, but also the detection of the epigenetic status of the sequenced DNA.

Results: The activity of these enzymes is associated with plasmid replication and depends on the origin of replication. While ColEI and p15 origins support plasmid single-strand modification, the pSC101 origin does not. Moreover, these enzymes work as a complex with DNA polymerase I that is required for MTase activity. It is possible they control plasmid and phage replication by discriminating DNA polymerase I-dependent and non-dependent plasmid origins. Also we suggest that the base flipping inherent to DNA modification may allow the MTase to perform a DNA helicase function and thereby help to control the rate of DNA polymerization to prevent excessive recombination.

PM140 Visualization of fundamental differences between phages and bacteria regarding phylogenomics

Matthias Hoetzinger[1], Emelie Nilsson[1], Karin Holmfeldt[1]

[1]*Linnaeus University, Department of Biology and Environmental Science, Sweden*

Background: Genome sequencing has revealed that there is no conserved gene universally present in all phages. To infer phylogenies, phages need to be clustered into groups that share at least some common genes. Phylogenomics is more straightforward for bacteria, since 92 genes have been identified that are present in most sequenced genomes. Comparative genomics is particularly useful for delineating bacterial species, for which a gold standard of 95% average nucleotide identity (ANI) has emerged. Recently, similar thresholds have been proposed to differentiate groups of phages.

Objectives: We aimed to visualize how diversity of phages is structured in comparison to bacteria. Thereby, we quantified genomic relatedness within currently defined taxonomic ranks and examined the feasibility of genome-based thresholds to delineate phage taxa.

Methods: We applied comparative genomics methods to publicly available phage and bacterial genomes, and more than 100 in-house phage genomes. We analyzed how the aligned fraction of genomes (AF) decreases with ANI. Moreover, we calculated phylogenomic trees and characterized pan- and core genomes at different levels of relatedness.

Results: While the relation of AF and ANI was comparable for phages and bacteria at high nucleotide identities (>95%), the decline of AF was disproportionately steep for phages towards lower ANI values. From a comparative genomics perspective, a similar threshold as is commonly used for the delineation of bacterial species might be conceivable to differentiate phage species. At higher taxonomic ranks, phylogenomic comparisons refer to only minor fractions of the genomes, and thus, hardly inform about the relatedness between different taxa.

PM141 Complete genome sequencing and physiological characterisation of the rare actinomycete Streptoalloteichus sp. NAI 85712

Emilia Palazzotto[1], Omkar Mohite[1], Margherita Sosio[2], Stefano Donadio[2], Kai Blin[1], Sang Yup Lee[3], Tilmann Weber[1]

[1]Technical University of Denmark , The Novo Nordisk Foundation Center for Biosustainability , Kongens Lyngby, Denmark
[2]NAICONS Srl, Milano, Italy
[3]Korea Advanced Institute of Science and Technology, Department of Chemical and Biomolecular Engineering , Yuseong-gu, Daejeon , Korea, Rep. of South

Background: Bacteria and in particular actinomycetes produce a notable array of bioactive molecules widely used as antibiotics. Due to the decrease of discovery of novel molecules from *Streptomyce*s, rare actinomycetes and rational metabolic engineering approaches represent a source of novel bioactive products with a broad range pharmacological activities.
Objectives: In our study the biosynthetic potential of *Streptoalloteichus* sp. NAI 85712 a rare actinomycetes was investigated by genomic analysis and physiological characterization.

1. Analysis of the biosynthetic potential of NAI 85712.
2. Physiological characterization of metabolic capabilities.

Methods:

1.
   - Whole-genome sequencing of NAI 85712 using PacBio and Illumina sequencing.
   - Genome mining.
2.
   - Biolog analysis

Results: Ribosomal 16S gene sequencing suggests a close similarity between NAI 85712 and *Streptoalloteichus tenebrarius* (99%) the producer strain of apramycin carbamoyl tobramycin and kanamycin B. Whole-genome sequencing of NAI 85712 was performed. A genome mining analysis showed that NAI 85712 genome contains 29 biosynthetic gene clusters (BGCs), including two non-ribosomal peptide synthetase (NRPS) BGC, one type I polyketide synthase (PKS) BGC, two type II PKS BGCs, one type III PKS, two hybrid PKS-NRPS and three aminoglycoside BGCs. A hybrid NRPS-PKS showed 72% similarity to caerulomycin BGC in *Actinoalloteichus cyanogriseus* WH1-2216-6. The production of aminoglycosides and caerulomycin was detected by HPLC and by growth inhibition assay.
The availability of the genome sequence of the rare actinomycete NAI 85712 provides a framework for bio-technological analysis and characterization of new natural products.

PM142 Genetic mutations and DNA rearrangements associated with de novo antimicrobial resistance

Marloes Hoeksema[1], Tania Darphorn[2], Martijs Jonker[3], Benno ter Kuile[4]

[1]*University of Amsterdam, Swammerdam Institute Of Life Sciences, Amsterdam, Netherlands*
[2]*University of Amsterdam, Swammerdam Inistitute of Life Sciences, Amsterdam, Netherlands*
[3]*University of Amsterdam, Swammerdam Insititute Of Life Sciences, Amsterdam, Netherlands*
[4]*University of Amsterdam, Swammerdam Istitute of Life Sciences, Amsterdam, Netherlands*

Background: When *E. coli* is exposed to non-lethal concentrations of antimicrobials it rapidly develops *de novo* resistance. Stepwise increasing levels of antibiotics cause full resistance in 15 to 25 days.

Objectives: This line of research aimed to document all genetic modifications that appear in newly resistant strains and identify mutations common to different antimicrobials.

Methods: Duplicate strains of E. coli MG1655 wildtype were made resistant to amoxicillin, kanamycin, enrofloxacin or tetracycline. Duplicates of resistant strains were subsequently made resistant to the remaining three antibiotics, resulting in sixteen sets of four strains with identical exposure history. These were subjected to whole genome sequencing.

Results: Induced resistance is accompanied by point mutations and genome rearrangements that are specific for each antibiotic applied. Mutations for a secondary resistance differ from those for initial resistance to the same drug. Extensive genomic rearrangements occur as well, with various insertion sequence transpositions correlating with exposure to specific antibiotics. The excision of prophage e14 as a result of the SOS response was often observed in secondarily resistant strains. Most striking was the amplification of a DNA fragment including *ampC* coding for a beta-lactamase by a factor of approximately 55, but sometimes exceeding 100 as a result of exposure to amoxicillin. This fragment could be transferred to a beta-lactam sensitive recipient strain, which became resistant after uptake of this fragment. The overall conclusion is that a complex set of DNA mutations confers *de novo* resistance.

PM143 Genome-wide diversity and phylogeography of mycobacterium bovis in tuberculosis hotspot area

Ana Reis[1;2], Liliana salvador[3], ana botelho[2], Rogério tenreiro[4], Mónica cunha[1;2;4]

[1]Centre for Ecology, Evolution and Environmental Changes (cE3c), Faculdade de Ciências da Universidade de Lisboa, Lisboa, Portugal
[2]INIAV, IP- National Institute for Agrarian and Veterinary Research, Oeiras, Portugal
[3]University of Georgia, College of Veterinary Medicine, Department of Infectious Diseases, Georgia, United States
[4]Biosystems & Integrative Sciences Institute (BioISI), Faculdade de Ciências da Universidade de Lisboa, Campo Grande, Lisboa, Portugal

Background: Animal tuberculosis (TB), caused by *Mycobacterium bovis* (*M. bovis*), is a widespread infectious disease of livestock and wildlife, that has the potential to cause significant socioeconomic, biodiversity and public health impacts.

In Portugal, TB is maintained in a multihost system with epidemiological relevance in cattle, red deer and wild boar, however the specific role exerted by each species in transmission cascades is under scrutiny.

Objectives: The main aim of this study was to refine knowledge on *M. bovis* population diversity and structure and to better understand TB transmission dynamics.

Methods: A robust *M. bovis* (*n*=487) dataset isolated from cattle, red and wild boar, over a 14-year period, was characterized by classic genotyping techniques, and whole-genome sequencing (WGS) was applied in a defined sub-population set (*n*=44). Pathogen genomic data with spatial and temporal data were integrated in a Bayesian inference analysis framework.

Results: The first assessment of genetic structure, using classic genotyping tecnhiques, combine Minimum Spanning Trees and Bayesian analyses, identifying five hypothetical clusters with evidences of geographic specificity.

This genetic structure was further validated by WGS and single nucleotide polymorphisms variant calling, decomposing the sub-population into five main genetic clades. Phylogenetic analyses and ancestral state reconstruction allowed the reconstruction of transmission trees and phylogeographic inference , with indication of recent inter- and intra-species transmission.

These results provide new insight into *M. bovis* demographic history in Portugal, evidencing the role of wildlife species as key players in the spread of TB. This knowledge is crucial to inform new control choices.

PM144 Omics study of arsenic resistance genes and clusters

Yiren Yang[1], Jack Yang[2], tingting zhao[1], huangcan li[1], Yan-xia Lin[3], Ren Zhang[1]

[1]*University of Wollongong, School of Chemistry and Molecular Bioscience, Wollongong, Australia*
[2]*University of Wollongong, SMART, Wollongong, Australia*
[3]*University of Wollongong, School of Mathematics and Statistics, Wollongong, Australia*

Background: Arsenic is a common element on earth, known to be toxic and carcinogenic. To survive under arsenic stress, living organisms have developed various arsenic resistance (*ars*) pathways, involving various genes. Bacteria, commonly harboring *ars* genes and operons/clusters, have been extensively studied for their resistance mechanism. However, the *ars* clusters seem to be more diverse in organization and composition.

Objectives: A better understanding of *ars* genes/clusters is required to provide further insight into bacterial *ars* pathways.

Methods: Genome sequencing of highly resistant bacterial strains, mining of published genomic data to explore a large number of *ars* operons/clusters and protein profiling of a selected strain under arsenic stress were all carried out.

Results: Genome sequencing of *Ochrobactrum sp.* CDB2 and *Bacillus sp.* CDB3 (isolated previously) identified two novel *ars* operons. The proteomic study carried out on CDB3 indicated a co-operative regulation of central metabolism for the best survival under arsenic stress. A constant struggle against arsenic was noted, explaining the long-term adverse effect of arsenic on most living organisms. A global approach using currently identified *ars* genes in CDB2 and CDB3 on bacteria full genome database yielded numerous bacterial *ars* operons and revealed a much larger diversity of *ars* gene families. Previously uncharacterized and even unknown genes were noted for their possible function in arsenic tolerance. Further analysis of operon sequences has also assisted in discovering a novel regulatory mechanism for *ars* operon expression. The combined approach will help mapping out a comprehensive arsenic resistance pathway network in bacteria.

PM145 Functional Analysis of a Two-component system GacA/DJ41_1407 in Acinetobacter baumannii

Meng-Yun Wen[1], Guang-Huey Lin[1]

[1]*Master Program in Microbiology and Immunology, School of Medicine, Tzu Chi University, Hualien, Taiwan*

Background: *Acinetobacter baumannii* is a globally important nosocomial pathogen characterized by an evolving multidrug resistance. Among the signaling networks, the most predominant are two-component systems (TCSs). *In silico* analysis revealed that there are 17 regulators and 16 sensors in *Acinetobacter baumannii*, and only five of them have been characterized. In the genome organization, there are a sensor and regulator which gene function has not been characterized, *DJ41_1407* and *DJ41_1406* respectively.

Objectives: Make a comprehensive understanding of how GacA/DJ41_1407 regulates downstream genes in *Acinetobacter baumannii.*

Methods: *A. baumannii* Δ*gacA* and Δ*DJ41_1407* mutant strain were constructed by maker-less mutagenesis. Methods for determining the phosphorylation status of GacA were used Phos-tag™ Acrylamide and western blotting. The phenotypic test including bacteria growth curve, single carbon source culture, antibiotic resistant, stress response and motility. Gene regulation of GacA/DJ41_1407 was confirmed by transcriptome and qPCR.

Results: *In silico* analysis revealed that DJ41_1406 shared 60% amino acid sequence identity with GacA (global antibiotic and cyanide control) in *Pseudomonas fluorescens*. In this study, we named DJ41_1406 as GacA. In Phos-tag™ Acrylamide gel electrophoresis result indicated that GacA/DJ41_1407 is a pair of two-component system. The phenotypic test shows that GacA plays a role in bacteria growth, antibiotic resistant, stress response and alcohol metabolism. The transcriptome of wild type, Δ*gacA* and Δ*DJ41_1407* were already analyzed. GacA/DJ41_1407 positively regulate alcohol dehydrogenase and organic hydroperoxide resistant gene (*ohr*). In the future, I will make a comprehensive understanding of how GacA/DJ41_1407 regulates downstream genes that cause *A. baumannii* resistant to environmental stress.

PM146 Plasmids of nonpathogenic Listeria spp.

Cora Chmielowska[1], Dorota Korsak[2], Magdalena Szuplewska[1], Dariusz Bartosik[1]

[1]*Institute of Microbiology, Department of Bacterial Genetics, Warsaw, Poland*
[2]*Institute of Microbiology, Department of Applied Microbiology, Warsaw, Poland*

Background: The genus *Listeria* comprises of 20 species, including 2 pathogenic – *L. monocytogenes* and *L. ivanovii*. Numerous plasmids from *L. monocytogenes* strains have been identified and analyzed. However little is known about plasmids from other *Listeria* species (only 4 complete nucleotide sequences available in GenBank). These plasmids can potentially be a reservoir of antibiotic, disinfectant and heavy metal resistance genes.

Objectives: The aim of our study was to identify, characterize and perform comparative analysis of plasmids from nonpathogenic bacteria of the genus *Listeria*. This allowed us to better estimate the contribution of these replicons to the dissemination of resistance phenotypes among *Listeria* spp.

Methods: 240 nonpathogenic *Listeria* strains (isolated from food processing, urban and natural environments), representing 5 species (*L. seeligeri, L. innocua, L. welshimeri, L. grayi, L. marthii*), were analyzed for the presence of plasmids. The complete nucleotide sequences of the plasmids were obtained and analyzed for the presence of resistance genes. Phenotypic tests for antibiotic, disinfectant and heavy metal resistances of plasmid-containing strains were performed to verify if the identified resistance genes were functional.

Results: Several new plasmids were identified among the analyzed nonpathogenic *Listeria* strains, including unique plasmids from *L. seeligerii* strains, i.e. a species for which no complete plasmid sequence is available in GenBank. Plasmids isolated from food processing environments showed a high level of similarity to known *L. monocytogenes* replicons, while plasmids isolated from natural environments were more divergent. Many plasmids carried numerous transposable elements and functional resistance determinants.

PM147 Phylogeny of Ureaplasma diversum based on the glycosyltransferase gene sequences

Izadora de Souza Rezende[1], Maysa Barbosa[1], Lucas Miranda Marques[2], Jorge Timenetsky[1]

[1]*Universidade de São Paulo, Instituto de Ciências Biomédicas - Departamento de Microbiologia, São Paulo, Brazil*
[2]*Universidade Federal da Bahia, Instituto Multidisciplinar em Saúde, Vitória da Conquista, Brazil*

Background: Ureaplasma diversum is a pathogen of cows that may cause intense inflammatory responses in the reproductive tract and interfere with bovine reproduction. Glycoglycerolipids, synthesized by glycosyltransferases, are key structural membrane components in many mycoplasmas. The recent sequencing of the genome of U. diversum allowed to improve understanding of the molecular biology of these infections.

Objectives: The aim of the present study was determine the relationship between U. diversum reference strain and clinical isolates by sequence analysis of glycosyltransferase gene.

Methods: *U. diversum* ATCC 49782 and 54 clinical isolates were used. PCR was performed to identify the glycosyltransferase gene. The primers for amplification of the gene of interest were obtained based on the genomic nucleotide sequence of U. diversum ATCC49782 deposited in GenBank. The amplicons were sequenced and a phylogenetic tree was constructed with MEGA-X. Sequence of reference strain was analyzed using bioinformatics techniques.

Results: After the sequencing, the samples formed groups according to the genetic proximity between them, comprising samples originated from the same collection site. The bioinformatics analyses reveal that coding sequence of the UD216 gene from ATCC49782 has some amino acids inserted into the cytoplasmic membrane, with regions of good accessibility and antigenicity, despite the absence of signal peptide. This is the first study to analyze *U. diversum* glycosyltransferase sequences. We have shown that this gene can reveal details of the relationships between U. diversum isolates. It also supports advances in understanding the molecular biology of ureaplasma infections, promoting the development of new diagnostic and prevention alternatives.

PM148 Adaptive immune systems of uncultured microbial lineages across the tree of life

Ian Rambo[1], Tanner Wiegand[2], Blake Wiedenheft[2], Brett Baker[1]

[1]*The University of Texas at Austin, Marine Science, Port Aransas, United States*
[2]*University of Montana, Microbiology and Immunology, Bozeman, United States*

Background: The tree of life is rapidly expanding through the recovery of novel, uncultured microbial lineages via metagenomics. The mechanisms of immunity by which these microbes defend against viruses and mobile genetic elements (MGEs) has yet to be fully explored among all the new branches of life. Clustered, Regularly-Interspaced Short Palindromic Repeats (CRISPRs) and their associated genes (*Cas*), referred to as CRISPR-Cas systems (CCSs), provide archaea and bacteria with adaptive immunity capable of targeting any invading DNA or RNA. CCSs are comprised of repeat arrays separated by unique virus or MGE-derived spacers (CRISPRs) and multiple CRISPR-associated genes (*Cas*) involved in cleavage and incorporation of invading genetic material into the CRISPR array to add immune "memory." Additionally, non-*Cas* genes present in CCS loci can play accessory roles in immune responses. These systems exhibit incredible functional and structural diversity due to the rapid evolutionary arms race against viruses.

Objectives: Here we set out to determine the diversity and functional potential of CCSs and their predicted accessory genes across the tree of life.

Methods: We developed a bioinformatics pipeline to identify and classify putative CCSs and accessory genes. We mined CCS loci from over 32,000 genomes including novel lineages recovered from estuaries and deep-sea (Guaymas Basin, Gulf of California) sediments.

Results: This work provides a comprehensive view of CCS type distribution across an updated tree of life. The predicted functions of these newly-described CCSs and accessory genes give insight into the potential immune responses of uncultured microbial lineages.

PM149 Molecular characterization of new blaIND carbapenemases produced by Chryseobacterium indologenes

Ilaria Unali[1], Anna Bertoncelli[1], Edoardo Pavan[1], Marco Ligozzi[1], Annarita Mazzariol[1]

[1]*University of Verona, Department of Diagnostics and Public Health, Microbiology Section, Verona, Italy*

Background: *Chryseobacterium indologenes* is a Gram negative rarely pathogenic to humans. In the case of long-term patients, this bacterium can however be the cause of severe infections thanks to its ability to grow on numerous surfaces, forming biofilms and acting together with other bacterial species, increasing their resistance to antibiotic therapies. *C. indologenes* can show resistance to carbapenems, drugs considered last resource. Principal leaders of the resistance mechanism to this class of antibiotics are metal-beta-lactamases encoded by a *bla*IND gene, of which 15 different variants have already been deposited.

Objectives: The study focused on the molecular characterization of carbapenemases produced by *C. Indologenes* strains isolated during multi-drug resistant screening program in Verona University Hospital.

Methods: Five *C. Indologenes* strains carbapenem resistant were investigated with phenotypic tests (MBL E-test and rapid carbaNP test) to show carbapenemase production. Degenerate internal primers, were designed based on the similarities between the various IND sequences deposited in the GenBank. The obtained amplicons were sequenced, and new external degenerate primers were designed in order to obtain the full gene sequence.

Results: The five strains were confirmed carbapenemase producers. Based on the results of the sequence alignment MDR83 strain harbor the IND variant while MDR2317 strain the IND2 variant. The MDR 4817 strain produce a variant close to IND2 with L34N mutation. Strains MDR1204 and MDR1873 showed the same sequence that share an 85.1% identity with a carbapenemase gene belonging to IND family produced by *C. gleum*. These data confirm a high heterogeneity of IND family.

PM150 Genomic profile of Brazilian Methicillin-resistant Staphylococcus aureus resembles clone dispersed worldwide

Jessica Bomfim Almeida[1], Suzi Pacheco de Carvalho[1], Guilherme Barreto Campos[1], Leandro Martins de Freitas[1], Lucas Miranda Marques[1]

[1]Universidade Federal da Bahia (UFBA) - Campus Anísio Teixeira, Microbiologia, Vitoria da Conquista, Brazil

Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) is an emerging bacterial pathogen that causes a wide variety of diseases in humans. Comparative genomic analysis of strains may help better understand the wide diversity of their genetic profiles.

Objectives: The objective of this study was to analyze the genomic features of the resistome and virulome of Brazilian MRSA isolates and their relationship to other Brazilian and international MRSA strains.

Methods: The whole-genome of three MRSA strains previously isolated in Vitória da Conquista were sequenced, assembled, annotated and compared with another 49 MRSA genomes. A phylogenetic tree was constructed and the pangenome, and accessory and core genomes were built. The resistome and virulome of all strains were identified.

Results: Phylogenetic analysis of all 49 strains indicates different clones showing high similarity. The pan-genome of the analyzed strains consisted of 4,484 genes, being 31% of the genes part of the core genome, 47% part of the accessory genome and 22% singletons. It was possible to observe that each strain type is characterized by a distinct consensus repertoire of virulence genes. In the resistome analysis, the strains showed multiresistance, with the most recurrent genes conferring resistance to beta-lactams, fluoroquinolones, aminoglycosides and macrolides. Our results allowed broad comparisons of clinical isolates and information about diversity of MRSA strains. In addition, the genomic study of these isolates is relevant, as this is the first study with genomic analysis comparing strains of MRSA from Brazil to strains of MRSA from other parts of the world.

PM151 Intrastrain genetic variability within infecting treponemal populations differ between syphilis and yaws treponemes

Vojtěch Bartoň[1], Denisa Maděránková[1], Juraj Bosák[2], Markéta Nováková[2], David Smajs[2]

[1]Brno University of Technology, Department of Biomedical Engineering, Brno, Czech Republic
[2]Faculty of Medicine, Masaryk University, Department of Biology, Brno, Czech Republic

Background: Pathogenic treponemes related to syphilis-causing treponeme comprise both human and animal pathogens. Human pathogens comprise three subspecies causing different diseases in humans and include *Treponema pallidum* ssp. *pallidum* (TPA, the causative agent of syphilis), *T. p.* ssp. *pertenue* (TPE, the causative agent of yaws), and *T. p.* ssp. *endemicum* (TEN, the causative agent of bejel).

Objectives: The main aim of this study was to analyze intrastrain genetic variability within infecting treponemal populations of the strains belonging to TPA, TPE and TEN subspecies.

Methods: This study was performed on a preselected treponemal genomes comprising both TPA and TPE strains and one genome of TEN. The following criteria were used to enroll genomic data into this study: i) available complete genome sequences and ii) average depth sequencing coverage over 100x.

Results: We have identified over 150 unique sites showing intrastrain heterogeneity. A substantial portion of identified sites showing genetic heterogeneity were found exactly at the same positions in two and more genomes with the highest number of 4 genomes with the same heterogeneity. Unlike previous studies, this study revealed heterogeneous sites also within TPE strains with a similar extent like in TPA strains. Interestingly, those sites were different as shown by phylogenetic trees constructed from these sites. This finding indicates an important genetic difference between TPA and TPE strains.

PM152 Trade-off between meiotic recombination and epigenetic potential

Ioannis Papaioannou[1], Nicolas Delhomme[2], Vasileios Vangalis[3], France A. Peltier[1], Lars M. Steinmetz[4;5;6], Michael Knop[1;7]

[1]University of Heidelberg, Zentrum für Molekulare Biologie der Universität Heidelberg (ZMBH), Heidelberg, Germany
[2]Swedish University of Agricultural Sciences, Umeå Plant Science Centre, Department of Forest Genetics and Plant Physiology, Umeå, Sweden
[3]National and Kapodistrian University of Athens, Department of Genetics and Biotechnology, Faculty of Biology, Athens, Greece
[4]European Molecular Biology Laboratory (EMBL), Genome Biology Unit, Heidelberg, Germany
[5]Stanford University School of Medicine, Department of Genetics, CA, United States
[6]Stanford University, Stanford Genome Technology Center, Palo Alto, CA, United States
[7]German Cancer Research Center (DKFZ), DKFZ-ZMBH Alliance, Cell Morphogenesis and Signal Transduction Group, Heidelberg, Germany

Background: The ubiquity of eukaryotic sex despite its significant costs has been a persistent conundrum in biology. Its paradoxical evolutionary success is largely attributed to meiotic crossing over, which is thought to handle mutation loads whilst ensuring faithful chromosome segregation in meiosis.

Objectives: To establish and employ the yeast species *Saccharomycodes ludwigii* –which experiences a full sexual cycle without any crossing over– as an ideal organism for revealing novel insights into genome evolution in the absence of meiotic recombination.

Methods: Genome sequencing and assembly; comparative genomics analyses of meiotic gene content and repetitive/low-complexity elements with 100 sequenced Saccharomycetes and other fungi; high-resolution DNA variant segregation analysis in meiosis; mutation accumulation analysis; functional study of important meiotic genes and experimental characterisation of putative prion-like proteins.

Results: Crossing over was confirmed to be absent from meiosis in *S. ludwigii*. Sequencing and analysis of its genome revealed unusually frequent AT-rich low-complexity stretches (genomic AT-content: 69.2%), both in intergenic and gene-coding regions. Overrepresented AT-rich tandem codon repeats within genes were predicted to be often translated to homopolymers or low-complexity amino-acid regions, such as poly-N repeats. This results in a significant expansion of prion-like domains in the *S. ludwigii* proteome, especially in proteins involved in transcriptional control and signal transduction. Selected prion-like candidates were indeed shown to form cytoplasmic aggregates upon overexpression, which propagated in dividing cells by migrating through buds. This study provides novel insights into the evolutionary trade-off between meiotic recombination and epigenetic factors as alternative sources of adaptive potential.

PM153 Analysis of the Dickeya genus diversity

Frédérique Van Gijsegem[1], Jacques Pédron[2]

[1]*INRA, Institute of Ecology and Environmental Sciences of Paris*
[2]*sorbonne-université, Institute of Ecology and Environmental Sciences of Paris*

Background: The *Dickeya* genus comprises aggressive soft rot plant pathogens with wide geographic distribution and host ranges. It includes ten species, seven of them (*D. chrysanthemi, D. dadantii, D. dianthicola, D. fangzhongdai, D. solani, D. paradisiaca, D. zeae)* grouping causative agents of maceration-associated diseases that impact a wide variety of crops and ornamentals, including both monocots and dicots. Members of several of these species were also isolated from water sources. The three remaining species (*D. aquatica, D. lacustris, D. undicola*) have recently been isolated only from water sources, so far. Here, we analysed the *Dickeya* genetic diversity in relation to species affiliation and habitats.

Objectives: Our goal was to analyse the possible links between genomic characteristics and diversity in habitats or geographical origin by exploring the signature genetic traits of the species and the intra-species variabilities.

Methods: We compared the genomes of 60 strains belonging to the ten *Dickeya* species, isolated from various hosts and from different environments, to determine genus and species core and pan-genomes and identify species- and strain-specific genes.

Results: This study revealed the large conservation of virulence-associated genes in all species (including "water-specific" ones), identified the genome-specific traits of the various *Dickeya* species and highlighted the intra-species diversities. These diversities encompass secondary metabolite biosynthetic pathways and toxins or the repertoire of genes of extrachromosomal origin as well as metabolic specificities. It also questions the affiliation of the *D. aquatica/D. lacustris/D. paradisiaca* species clade to the *Dickeya* genus. Links between genomic characteristics and habitats will be discussed.

PM154 Genome analysis of a novel strain Psychrobacillus sp., isolated from an iceberg

Jun Young Choi[1], Kunjoong Lee[1], Pyung Cheon Lee[1]

[1]Ajou University, Department of Molecular Science and Technology and Department of Applied Chemistry and Biological Engineering, Sowon, Korea, Rep. of South

Background: A psychrotolerant strain *Psychrobacillus* sp. PB01 was isolated from the Antarctic iceberg near the King Sejong Station.

Objectives: The genome of the novel strain *Psychrobacillus* sp. PB01 was completely sequenced and annotated to develop genetic engineering tools useful for manipulating *Psychrobacillus* strains.

Methods: The genomic DNA of *Psychrobacillus* sp. PB01 was extracted and sequenced in SMRT cells using the Pacific Biosciences RS II single-molecule real-time sequencing technology kit. Genome assembly and annotation were carried out by using the RAST server online, Prokka, Glimmer, tRNAscan-SE, RNAmmer, RPS-BLAST and BLASTp softwares. Genome maps of the chromosome and the plasmid were generated using Circos software.

Results: The genome of *Psychrobacillus* sp. PB01 consists of a single circular chromosome (4,332,095 bp) and a plasmid (19,243 bp). The chromosome has 4,377 coding DNA sequences (CDS) and 110 RNAs (33 rRNA + 77 tRNA). The higher number of copies of rRNA and tRNA genes suggests that fast cellular responses of the strains PB01 to environmental changes. The plasmid has 28 CDSs. The plasmid could replicate via a rolling-circle replication mode.

PM155 Comparative genomics of staphylococcus aureus from bovine mastitis

Daesung Ko[1], Jae-Hong Kim[1], Hyuk joon Kwon[2], Se hee Ahn[1;2], Namhyung Kim[1;2], Jin mi Park[1], Eun-Jin Ha[1], Seung-Min Hong[1]

[1]Seoul National University, Avian Disease Lab., Seoul, Korea, Rep. of South
[2]Seoul National University, Korea, Rep. of South

Background: *Staphylococcus aureus* is the major pathogen causing bovine mastitis globally and livestock-associated methicillin resistant *S. aureus* (LA-MRSA) has become a potential threat to public health. MRSA from bovine mastitis is not frequent but a methicillin susceptible *S. aureus* (MSSA) genotype, *rpoB* sequence type (RST)10-2 (RST10-2), is prevalent in Korea. To date, many genomic sequences of *S. aureus* have been elucidated, but complete genome sequences of RST10-2 MSSA from bovine mastitis have never been reported.

Objectives: We compared the entire genome of the RST 10-2 MSSA strains to provide hints to delineate the evolutionary steps of the human ancestor of RST10-2 during bovine adaptation.

Methods: We determined the complete genome sequences of two RST10-2 MSSA that differ from each other in staphylococcal protein A and molecular prophage types [PMB64-1 (t2489/mPPT0) and PMB81-4 (t127/mPPT1-2-3)] and conducted a comparative genomics study.

Results: The genomic sequences of PMB64-1 and PMB81-4 were more homologous to the representative human RST10-2 strains (MSSA476, MW2 etc.) compared to other RSTs; Most of them shared common five pseudogenes, along with high amino acid identity of four variable virulence genes that were identified in this study. However, PMB64-1 and PMB81-4 acquired different strain-specific pseudogenes and mobile genetic elements from human strains. The unique pseudogene profile and high identity of the virulence genes were verified in RST10-2 field isolates from bovine mastitis. Thus, bovine mastitic RST10-2 MSSA may have an evolutionary relationship with the human RST10-2 community-associated (CA) MSSA and CA-MRSA strains but may have adapted to cows.

PM156 Comparative genomics and deep phenotyping of the plant-associated genus Ensifer

Camilla Fagorzi[1], Carlo Viti[2], Francesca Decorosi[2], Alezandru Ilie[1], Alessio Mengoni[1], George diCenzo[1]

[1]*Via Madonna del Piano, 6, Dept. of Biology, Sesto Fiorentino, Italy*
[2]*University of Florence, Scienze e Tecnologie Agrarie, Alimentari, Ambientali e Forestali (DAGRI), Firenze, Italy*

Background: The *Rhizobiaceae* family of the Alphaproteobacteria includes species able to perform nitrogen fixation during symbiotic interactions with plants. However, not all species within this family present these abilities. In fact, horizontal transfer of symbiotic plasmids/islands has played a crucial role in the evolution of symbiotic phenotypes. Within the genus *Ensifer* (syn. *Sinorhizobium*), it is possible to find both symbiotic and non-symbiotic species.

Objectives: The aim of this work is to characterize the phylogenetic relationships inside the genus *Ensifer* by comparative genomics and Phenotype MicrorrayTM, in order to shed light on the different features of the symbiotic and non-symbiotic species.

Methods: In this study, comparative genomics and Phenotype MicroarrayTM were used to characterize different strain belonging to the genus *Ensifer*.

Results: Comparative genomics identified two distinct clades that clearly separate the symbiotic and non-symbiotic species. Genes involved in nodule formation and nitrogen fixation (*nodA, nodB, nodC, nifD, nifK, nifH*) were almost exclusively present in the "symbiotic" clade. The separation into two groups was also noted in the Phenotype MicrorrayTM data. In particular, the non-symbiotic species (originally isolated from soil and rhizospheres) showed higher resistance to harsh conditions (e.g. heavy metal) compared to the symbiotic organisms.

In conclusion, these preliminary data suggest that several genomic and phenotypic features are associated with the presence/absence of symbiotic phenotypes among species of the genus *Ensifer*.

PM157 Comparative Genomics of Listeria monocytogenes Isolated From Fresh Produce, Meat and Clinical Cases

Erin Lewis[1;2], J. Andrew Hudson[3], Nigel Cook[3], Jerry Barnes[1], Edward Haynes[2]

[1]*Newcastle University, IAFRI, Newcastle upon Tyne, United Kingdom*
[2]*Fera Science Ltd., United Kingdom*
[3]*Jorvik Food Safety Services, York, United Kingdom*

Background: *Listeria monocytogenes* accounted for 57% of deaths caused by foodborne disease in the EU in 2015, with contaminated food being the main route of infection. Whole genome sequencing of bacteria is increasingly being applied in the context of source tracking outbreak strains of *L. monocytogenes* and gives a wealth of genetic information.

Objectives: The purpose of this study was to examine the whole genome sequence of 128 isolates of *L. monocytogenes* isolated from fresh produce, meat products and clinical cases.

Methods: *L. monocytogenes* was isolated from fresh produce (n=48) and sequenced on the MiSeq. Meat (n= 34) and clinical (n=46) isolates whole genome sequences were downloaded from the NCBI Sequence Read Archive. All were bioinformatically analysed using Nullarbor.

Results: The results showed all *L. monocytogenes* analysed were lineage I and II. Isolates from fresh produce, meat and clinical samples did not cluster phylogenetically based on sample type. The results confirmed the AMR gene *fosX* is ubiquitous amongst *L. monocytogenes* isolates, and *brcB* and *brcC* were found in four isolates, two from vegetable origin, one from meat origin and one from clinical origin. The virulome showed no genes present in clinical samples that were not also present in meat and vegetable isolates.

These results suggest isolates that can lead to human cases of Listeriosis can be isolated from meat and fresh produce, and currently *L. monocytogenes* is not a key route of transmission for AMR genes, due to the low levels of these genes found in all samples.

PM158 Multiple promoters drive expression of genes of SigD-regulon in Corynebacterium glutamicum

Hana Dostalova[1], Tobias Busche[2], Jiří Holátko[1], Lenka Rucká[1], Václav Štěpánek[1], Ivan Barvík[3], Jan Nešvera[1], Jörn Kalinowski[2], Miroslav Pátek[1]

[1]*MIKROBIOLOGICKY USTAV AV CR, Laboratory of Molecular Genetics of Bacteria , PRAGUE, Czech Republic*
[2]*University of Bielefeld, Center for Biotechnology, Bielefeld, Germany*
[3]*Faculty of Mathematics and Physics, Charles University, Institute of Physics, Prague, Czech Republic*

Background: *Corynebacterium glutamicum* is an important industrial producer of various amino acids and other metabolites. *C. glutamicum* harbors five sigma subunits of RNA polymerase belonging to Group IV (SigC, SigD, SigE, SigH, and SigM). The role of SigD consists in the control of cell wall integrity. The SigD regulon is involved in the synthesis of components of the mycomembrane which is part of the cell wall in *C. glutamicum*.

Objectives: We wanted to describe transcriptional regulation of the SigD-dependent genes and their activity during stress conditions.

Methods: We performed transcriptome sequencing and identification of transcription start sites with RNA isolated from *C. glutamicum*, in vitro transcription and measurements of promoter activity by two-plasmid assay to investigate the SigD-dependent genes.

Results: RNA sequencing of the transcriptome from a strain overexpressing the *sigD* gene provided 29 potential SigD-controlled genes and enabled us to precisely localize their transcriptional start sites. Analysis of the respective promoters by both in vitro transcription and the in vivo two-plasmid assay confirmed that transcription of 11 of the tested genes is directly SigD-dependent. The key sequence elements of all these promoters were found to be identical or closely similar to the motifs -35 GTAACA/G and -10 GAT. We discovered separated or overlapping SigA-, SigB- or SigH-regulated promoters within the upstream region of 8 genes of the SigD-regulon. Multiple promoters active with different sigma factors ensure that the cells express the genes appropriately under both standard and stress conditions.

PM159 Metabolic reconstruction of hydrocarbon catabolic pathways of the hydrocarbonoclastic Achromobacter sp. strain B7

Flavia Dorochesi [1], Valentina Mendéz[1], Roberto Durán[1], Lisette Hernández[1], Francisco Salvà-Serra[2], Edward R. B. Moore[2], Michael Seeger[1]

[1]*Federico Santa María Technical University, Department of Chemistry & Centro de Biotecnología Daniel Alkalay Lowitt, Valparaíso, Chile*
[2]*Gothenburg University, Department of Infectious Diseases & Culture Collection, Gothenburg, Sweden*

Background: Pollution by oil spills has been reported in Chile. Bioremediation has been applied to clean-up hydrocarbon-polluted soils. *Achromobacter* species are Gram-negative bacilli commonly found in soil and water but also are associated with human clinical samples. *Achromobacter* sp. B7 is a bacterium isolated during bioremediation trials by bioaugmentation of a diesel-spiked soil from Valparaiso Region, Chile.

Objectives: The aim of this study is the metabolic reconstruction of hydrocarbon degradation in *Achromobacter* sp. B7.

Methods: Strain B7 was grown on hydrocarbons as sole carbon source. The genome was sequenced on an Illumina HiSeq 4000 system and Nanopore MinION instrument, assembled with SPAdes v.3.11.1 and annotated using PROKKA software. The identification of catabolic genes and their genomic context was carried out through homology-based analysis using Blast tool. Ring-hydroxylating oxygenases α-subunits were identified using profile hidden Markov models.

Results: Strain B7 grew on hexane, octane, hexadecane, naphthalene, biphenyl, and diesel as sole carbon sources. The hybrid assembly resulted in a closed and complete chromosome sequence of 6,236,552 bp with a G+C content of 64.8%. 5526 coding sequences and 65 tRNA were identified. Strain B7 possessed complete catabolic pathways of aliphatic hydrocarbons (*alk* genes). Genes involved in the catabolism of aromatic hydrocarbons were annotated in strain B7 genome. A high number of peripheral (*e.g.*, salicylate) and central pathways (*e.g.*, gentisate, catechol) were identified. The classification of the α-subunits of ring-hydroxylating oxygenases of strain B7 was carried out. This study indicate that strain B7 harbors a wide range of genes involved in hydrocarbon degradation.

PM160 Impacts of yqiC on the global transcriptome of Salmonella and interaction of YqiC with Salmonella colonization in human intestinal epithelium

Shiuh-Bin Fang[1;2], Chih-Hung Huang[3], Pei-Ju Chang[1;2], Hung-Hao Fan[1;2], Wei-Sheng Sun[1;2]

[1]Shuang Ho Hospital, Taipei Medical University, Division of Pediatric Gastroenterology and Hepatology, Department of Pediatrics, New Taipei City, Taiwan
[2]Taipei Medical University, Department of Pediatrics, School of Medicine, College of Medicine, Taipei City, Taiwan
[3]National Taipei University of Technology, Graduate Institute of Biochemical and Biomedical Engineering, Taipei City, Taiwan

Background: We reported that *yqiC* is required for colonization/invasion of *Salmonella* Typhimurium (*S*. Typhimurium) in human cells.

Objectives: To investigate how *yqiC* regulates other *Salmonella* genes and YqiC affects bacteria colonization.

Methods: First, *S*. Typhimurium *yqiC*-deleted mutant (Δ*yqiC*) and its wild-type strain SL1344 infected Caco-2 cells for 2h (MOI=50, n=3). Bacterial RNAs were isolated for RNA-sequencing (RNA-seq) and their global transcriptomes were compared. Second, Caco-2 cells were treated with YqiC/non-YqiC for 90m (MOI=5, n=4) before 90-m treatment of SL1344 (exclusion assay), 90-m co-incubation with SL1344 and subsequent 90-m incubation or not (competition assay), and after the 90-m treatment of SL1344 (displacement assay). SL1344 colonizing Caco-2 cells were quantified and compared between both groups.

Results: First, RNA-seq identified 117 significantly upregulated and 291 significantly downregulated genes (Δ*yqiC*/SL1344 ratio>log2 and <-log2 fold-change, $p<0.005$). *yqiC* is involved in *Salmonella* pathogenesis, pilus assembly, fimbiral usher porin activity, response to antibiotic, and transaminase activity (Emapplot of GO analysis). *yqiC* regulates ion-sulfur cluster assembly, iron ion binding, molybdenum ion binding, molybdopterin cofactor binding, and de novo inosine monophosphate biosynthetic process (Cnetplot of GO analysis). KEGG analysis showed the genes involved in *Salmonella* invasion, the phosphotransferase system, the two-component system, alanine/aspartate/glutamate metabolism, glycolysis/gluconeogenesis, and carbon metabolism. Second, YqiC significantly excludes ($p=0.041$) and displaces ($p=0.019$) *S*. Typhimurium from Caco-2 cells, but not competitively inhibiting bacterial colonization in Caco-2 cells. In conclusion, we discovered unreported *Salmonella* genes considerably regulated by *yqiC*. YqiC excludes and displaces *S*. Typhimurium from Caco-2 cells. The results provide clues for developing strategies against salmonellosis.

PM161 Improved Methods for Next Generation Sequencing Library Clean Up and Size Selection

Chris Moreland[1]

[1]*Promega, Madison,*

Background: NGS libraries require high quality nucleic acid inputs of varying quantity, concentration, and size depending on the library preparation methods and sequencing platforms used. Most methods use a magnetic bead-based chemistry throughout the overall protocol, with usage falling into two basic categories of function: Sample clean up to remove unwanted components and size selection to remove nucleic acid fragments or library molecules that are outside an optimal size range for downstream sequencing.

Objectives: Comparisons between commercially available size-selection beads were performed to assess library yield, reproducibility, size-selection precision, and final sequencing results. Whole genome sequencing was performed using the NEBNext Ultra II library prep kit on a range of DNA inputs from 1ng - 1µg. Size selection steps were executed with in parallel with both size-selection chemistries, followed by Illumina sequencing.

Methods: DNA yields increased 20% or more per sample cleanup step cycle and substantial reduction of undesired HMW DNA from libraries was achieved. Viscosity and bead response time are both reduced at least 5-fold, resulting in greater reproducibility and 2 to 4-fold reduction in library yield standard deviation. Sequencing results demonstrating duplication rates, coverage uniformity and reproducibility will be presented

Results: Improved DNA recovery from NGS library size-selection enabled reduction in starting sample size and reductions in PCR cycling, resulted in fewer duplicate reads, and greater coverage uniformity. All these enhancements can serve to improve any sequencing experiment, but are especially important when sequencing limited, heterogeneous samples such as those derived from FFPE or circulating cell-free DNA.

PM162 The first comparison of the European strain of Borrelia miyamotoi with strains from Asia and North America

Markéta Nováková[1], Petra Pospíšilová[1], Jan Janeček[1], Li Dantong[2], Ana Silva[3], Anita Cunha[3], Martin Starý[4], Eva Šmerdová[4], Alena Žákovská[5], David Smajs[1]

[1]*Faculty of Medicine, Masaryk University, Department of Biology, Brno, Czech Republic*
[2]*Southern Medical University, Guangzhou, China*
[3]*Faculty of Medicine, University of Porto, Porto, Portugal*
[4]*Faculty of Science, Masaryk University, Department of Botany and Zoology, Brno, Czech Republic*
[5]*Faculty of Science, Masaryk University, Department of Experimental Biology, Brno, Czech Republic*

Background: Borreliae are divided into the Lyme disease (LD) and the relapsing fever (RF) groups. *Borrelia miyamotoi* is the only member of the RF group transmitted by *Ixodes* spp. The whole genomes of *B. miyamotoi* from Asia and North America have been determined so far.

Objectives: The aim was to amplify DNA of *B. miyamotoi* from ticks and eggs of *I. ricinus* from the Czech Republic, as eggs contain a relatively high ratio of borrelial to tick DNA, which is suitable for the whole genome sequencing (WGS).

Methods: Altogether, 469 ticks were collected by flagging, and additional 267 engorged females were obtained from dogs and cats, from which 158 (59.2%) laid eggs. The *B. miyamotoi glpQ* gene was found in 10 (2.1%) questing ticks and 3 (1.9%) egg clusters. The sequences of 8 intergenic spacers (IGS) were compared within the dataset and tested with segments obtained from Asian and North American strains by Maximum Likelihood method (*Borrelia hermsii* as outgroup).

Results: The analysis of 3566 nucleotides of 8 IGS (514, 394, 483, 383, 473, 451, 467 and 401 nucleotides) revealed that Czech samples were identical to each other, and 6 out of 8 phylogenetic trees derived from IGS showed that Asian and North American strains are closely related to each other than to the Czech strain. This may be due to different tick host species in each geographical region. The WGS analyses will help to describe evolutionary history of *B. miyamotoi*.

PM163 Increased knowledge of magnetotactic bacteria diversity through the analysis of metagenomic data

Denis Grouzdev[1], Maria Uzun[1;2], Maria Krutkina[1], Lolita Alekseeva[1;2], Veronika Koziaeva[1]

[1]*Institute of Bioengineering, Research Center of Biotechnology of the Russian Academy of Sciences, Moscow, Russian Federation*
[2]*Lomonosov Moscow State University, Moscow, Russian Federation*

Background: Magnetotactic bacteria (MTB) are microorganisms that synthesize magnetosomes. Magnetosome synthesis is genetically controlled and requires specific magnetosome membrane genes. Only about 60 MTB genomes are known, and knowledge of their diversity remains negligible. Metagenomic data are constantly accumulating, although for the most part the data are not assembled into complete genomes. This work aimed to check for the presence of MTB in open databases.

Objectives: To check for the presence of MTB, 61410 bacterial genomes and 10587 in the IMG database were analysed.

Methods: The magnetosome-associated protein MamK was used for BLAST analysis. Metagenomes with MamK were binned using DAS Tool with preliminary binning with BusyBee, Maxbin2 and MyCC. Bins with MamK were checked for completeness, and contamination was checked with CheckM. The bins' phylogeny was determined using a concatenated set of 120 marker proteins. genes A phylogenetic tree of concatenated magnetosome associated proteins MamABIKMPQ was constructed.

Results: In the result, 231 MamK proteins were obtained from 139 metagenomic and 4 genomic DNA sequences. After the binning process, 140 bins containing MamK were found. The bins' phylogeny determination revealed for the first time the presence of MTB in the *Nitrospinae* phylum and in *Geobacteraceae* and *Syntrophobacteraceae* of Deltaproteobacteria. New MTB representatives were also found in groups where MTB was previously observed, such as the *Alpha-*, *'Eta'-*, *Delta-* and *Gammaproteobacteria* and the phyla *Nitrospirae*, *Planctomycetes* and *Omnitrophica*. A comparison of trees based on Mam proteins and on 120 core genome proteins allowed the reconstruction of the evolutionary pathways of MTB.

PM164 Molecular markers in ustilago esculenta to identify pregnant times in zizania latifolia

Xintao Ge[1], Wenqiang Xia[1], Yafen Zhang[1], Zihong Ye[1]

[1]*China Jiliang University, Hangzhou, China*

Background: Over 30,000 hm^2 of *Zizania latifolia* planted in Zhejiang Province, China. The pregnant period of *Z. latifolia* is concentrated in April-June and September-November every year. Early-pregnant and late-pregnant cultivated varietie can often occupy the golden period of market price of *Z. latifolia*, so as to improve the economic benefits of *Z. latifolia*. *Ustilago esculenta* which was a uniquely pathogenic fungus, could infect *Z. latifolia*, then suppressed host heading to flowering and triggered host stem swellen to produce Jiaobai, so we speculate that the characteristics of *U. esculenta* may affect the characteristics of *Z. latifolia*. SNP marker is considered to be an effective and accurate marker because of its large amount of data and the universality.

Objectives: The development of early-pregnant and late-pregnant molecular markers can be directional breeding of *Z. latifolia* varieties, to overcome the traditional breeding process of tedious, long cycle, huge investment and other shortcomings.

Methods: We have sequenced and assembled the genome of *U. esculenta*, so we found a large number of SNPs and developed SNP molecular markers related to the pregnant time of *Z. latifolia* by re-sequencing the genomes of *U. esculenta* from *Z. latifolia* at different pregnant time. Its verification methods, including allele specific PCR (AS-PCR) and high-resolution melting (HRM) method.

Results: Verification of *U. esculenta* of 48 species of *Z. latifolia.* We have developed eight molecular markers related to the early and late pregnant time of *Z. latifolia*.

PM165 Characterization of actP is regulated by a two-component system EmaSR in A. baumannii

Yu-Tien lin[1], Guang-Huey Lin[2]

[1]*Tzu Chi University, Department of Molecular Biology and Human Genetics, Hualien, Taiwan*
[2]*Tzu Chi University, Master Program in Microbiology and Immunology, Hualien, Taiwan*

Background: The serious medical problem caused by *Acinetobacter baumannii* could be attributed to it can use the low concentration of ethanol as a carbon source. In the previous study, our lab found the sensor kinase EmaS and the response regulator EmaR are related to the ability of ethanol metabolism in *A. baumannii*, and due to transcriptome data, we found *DJ41_3174* (*actP)* and *DJ41_3173* have been high upregulated by two-component system EmaSR.

Objectives: Characterization of *actP* is regulated by a two-component system EmaSR in *A. baumannii*.

Methods: We confirm *emaSR* mutants and complementary strains related to the ability of alcohol metabolism by sole carbon source culture. To investigated the domain functions of EmaS and EmaR by using the database. We already constructed *actP* and *DJ41_3173* double mutant by marker-less mutagenesis. We will construct the expression strain to study the phenotypes and the function of ActP and DJ41_3173.

Results: EmaSR plays an important role in ethanol metabolism. According to transcriptome data, EmaSR upregulation 30 genes belong to the transport protein, and 25 genes belong to metabolism. EmaS consists of a histidine kinase, and EmaR consists of receiver domain and helix-turn-helix (HTH) DNA-binding motif. Acetate permease (ActP) belongs to the sodium: solute symporter family, we found ActP related to the ability to use acetic acid as a carbon source. In the future, we will confirm the function of ActP and DJ41_3173.

PM166 Comprehensive phylogenetic analysis and genome dynamics of O-serogroup reference strains in Vibrio cholerae

Kazunori Murase[1], Masatomo Morita[2], Taichiro Takemura[3], Eiji Arakawa[2], Hidemasa Izumiya[2], Ichiro Nakagawa[4], Taisei Kikuchi[1], Makoto Ohnishi[2]

[1]*University of Miyazaki, Department of Infectious Diseases, Japan*
[2]*National Institute of Infectious Diseases, Department of Bacteriology I, Japan*
[3]*Nagasaki University, Institute of Tropical Medicine, Japan*
[4]*Kyoto University, Department of Microbiology, Japan*

Background: *Vibrio cholerae* is a member of the Vibrionaceae family that is found in coastal waters and estuaries, and more than 200 serogroups have been identified. However, only two O-serogroups, O1 and O139, are associated with severe, life-threatening diarrheal disease cholera and responsible for its global pandemics. The epidemiological or genomic studies on O1 and O139 have been reported frequently, but the genetic diversity of *V. cholerae* population have yet to be elucidated and their understandings are limited.

Objectives: Here we performed a comprehensive genome analysis of O-serogroup reference *V.cholerae* strains to reveal the phylogenetic diversity and genomic characterization of *V. cholerae* population.

Methods: We determined the genome sequences of 206 O-serogroup reference strains and performed phylogenetic and pan-genome analysis for *V. cholerae* population. We further determined complete genome sequence of 10 *V. cholerae* strains to compare the localization of core genes between chromosome 1 and 2.

Results: Pan-genome analysis demonstrated that the *V. cholerae* pan-genome could be considered "open" with size increasing logarithmically. This result indicated the *V. cholerae* population increased the genetic diversity to acquire exogenous genes from the natural habitats. In addition, *V. cholerae* population can be phylogenetically separated into mainly 3 clusters with statistical significance. However, number of core genes identified in chromosome 1 and 2 was exactly same regardless of cluster, indicating no exchange event for any core genes between two chromosomes. Our results could be an important clue to give us a deep understanding of *V. cholerae* evolutionary processes.

PM167 Surveillance of beta-lactam, azithromycin and fosfomycin resistance in non-typhoidal Salmonella: Characterisation of an S. Infantis plasmid

Winnie Lee[1;2], David Greig[2;3], Gemma Langridge[4], Samuel Bloomfield[4], Alison Mather[4], Andrew Edwards[1], Marie Chattaway[2], Satheesh Nair[2]

[1]*Imperial College London, London, United Kingdom*
[2]*Public Health England , Gastrointestinal Bacteria Reference Unit, London, United Kingdom*
[3]*University of Edinburgh, United Kingdom*
[4]*Quadrum Institute, United Kingdom*

Background: Non-typhoidal *Salmonella* (NTS) infections are associated with high morbidity and mortality. β-lactams are used as first-line treatment but resistance to these has increased considerably in recent years. Azithromycin and fosfomycin are used as alternatives; however, the incidence of resistance in these drugs is also increasing.

Objectives: To quantify and characterise UK genomic and antimicrobial resistance surveillance data on NTS.

As multi-drug resistance (MDR) was particularly a concern in the *S.* Infantis population, long read sequencing was used to characterise a MDR *S.* Infantis isolate.

Methods: Epidemiological surveillance on 35,372 NTS received by Public Health England was conducted for analysis of demographics, including global travel.

An isolate of *S.* Infantis showing multi-drug resistance was sequenced on Oxford Nanopore's MinION.

Results: No isolates were resistant to β-lactams, azithromycin or fosfomycin alone but all isolates were resistant to multiple antimicrobial classes. IncHI2, IncY and IncN plasmids were predominantly found in the most multi-drug resistant NTS isolates.

In the sequenced *S.* Infantis we identified a megaplasmid (IncFIB) and found resistance determinants *fosA, arsA, arsD* and bla_{CTXM65}. Analysis of the IncFIB plasmid revealed 99% similarity to an IncFIB plasmid in an *S.* Infantis isolated from chickens in the USA. Horizontal gene transfer of AMR genes is the most likely cause of the increase in resistance genes detected within the *Salmonella* population in both humans and animals. Whole genome sequencing utilised in global surveillance of *Salmonella* isolates allows characterization of AMR determinants and prediction of emerging resistance profiles in the UK *Salmonella* population.

PM168 Functional characterization of genes in the cosmomycin D cluster in Streptomyces olindensis DAUFPE 5622

Luana Cunha[1], Leandro Garrido[1], Marcelo Pena[2], Gabriel Padilla[1]

[1]*Biomedical Sciences Institute, Biotecnology, São Paulo, Brazil*
[2]*Phytochemistry, Botanic, São Paulo, Brazil*

Background: The genus *Streptomyces* has been studied in recent decades for the ability to produce various bioactive compounds. The microorganism *Streptomyces olindensis* produces molecules known as cosmomycins, among which cosmomycin D is the major fraction. This antitumor antibiotic is a tetracyclic aromatic polyketide of the anthracycline family. Studies of production, toxicity, structural analysis, chemical properties, DNA-anthracycline interaction and biosynthesis have already been performed indicating the potential of cosmomycin to be used for the treatment of neoplasias. There are several studies aimed at improving the pharmacological properties of anthracyclines by modifying sugars.

Objectives: The aim of the work is to search for analogues of natural polyketide products with novel or improved pharmacological properties through genetic modifications in the biosynthesis process of the molecule cosmomycin D.

Methods: The genes of *Streptomyces olindensis cosT* (WP_037757725.1 cytochrome P450), *cosT2* (WP_037757696 .1 hypothetical protein) and *cosG2* (WP_037757699.1 glycosyltransferase) are being studied to understand the involvement of sugars in the activity of the molecule. By means of molecular biology techniques, null mutants were obtained for these genes in *Streptomyces olindensis* to elucidate the aglycone glycosylation mechanism.

Results: HPLC analyzes showed a different profile compared to the wild strain, indicating the importance of these genes in the construction of the molecule. The understanding of the involved enzymatic mechanisms is important for the development of new compounds with improved therapeutic profile through combinatorial biosynthesis.

PM169 Bacterial Ig-like proteins expressed by IncHI resistance plasmids

Mario Huttener Queiroz[1], Alejandro Prieto[1], Manuel Bernabeu[1], Sonia Aznar[1], Estibaliz Glaria[2], Annabel Valledor[2], Jon Hergueta [1], Susana Merino[1], Juan Tomas[1], Antonio Juarez[1]

[1]Faculty of Biology, Department of Genetics, Microbiology and Statistics, Barcelona, Spain
[2]Faculty of Biology, Department of Cell Biology, Physiology and Immunology, Barcelona, Spain

Background: Antimicrobial resistance (AMR) is nowadays a major threat to public health. Dissemination of AMR among different bacterial populations occurs mainly due to the presence of plasmids that harbour the AMR determinants. The incompatibility group (Inc) IncHI is one of the groups of bacterial plasmids that confer AMR to several enterobacteria.

Objectives: To characterize a large molecular mass protein (termed RSP) that contains an Ig-like domain and that is detected in the external surfaces of the bacterial cells that harbour IncHI plasmids.

Methods: We constructed a knockout of the *rsp* gene and transcriptional fusions of *rsp* with a reporter gene. We also performed epitope tagging of the RSP protein.

Results: The protein is exported to the external medium by using the plasmid-encoded type IV secretion system that is also used for its transmission to the recipient cells. Expression of RSP protein reduces cell motility and enables plasmid conjugation. The RSP protein appears to be associated to the flagella. Bacterial proteins containing Ig-like domains and showing a significant degree of similarity to RSP play relevant roles in adherence. Most likely, the protein must facilitate cell-to-cell contact previous to conjugation. When proven to be antigenic, the RSP protein could be targeted in order to control IncHI plasmids dissemination in natural environments, or to combat infections caused by AMR enterobacteria that harbour IncHI plasmids.

PM170 Integrative Elements as drivers of genome plasticity in the marine cyanobacterium Prochlorococcus

Raphaël Laurenceau[1], Christina Bliem[1], Zev Cariani[1], Thomas Hackl[1], Markus Ankenbrand[1], Sallie Chisholm[1]

[1]*MIT, Civil and Environmental Engineering*

Background: *Prochlorococcus* is the smallest and numerically most abundant cyanobacterium in the oceans. They possess a large pan-genome containing hypervariable genomic islands that are linked to niche differentiation.

Objectives: Our objective is to understand how genomic islands form, how they function, and how they evolve within the *Prochlorococcus* pan-genome. This will help us unveil some of the evolutionary principles that shape the self-assembly and resilience of the global *Prochlorococcus* population 'collective'.

Methods: We performed a comparative genomics study of 600 *Prochlorococcus* genomes revealing the presence of integrative elements in their genomic islands. We then analyzed the transcription of 8 integrase genes in 4 different strains, and their dynamics inside genomes using quantitative PCR measurements of gene copy number.

Results: We have found that integrative elements are the primary driver of genomic island evolution in *Prochlorococcus*. They carry diverse sets of metabolic gene cargo that undergo rapid rearrangements among cells in culture. Most of them carry primase enzymes, and we show evidence of their ability to replicate within cells, offering a mechanism for the high recombination rates observed in island regions. Transcriptomic experiments revealed that most integrase enzymes are tightly regulated through the SOS response DNA repair pathway. Overall, our results suggest that genomic islands function as adaptive tools in the *Prochlorococcus* 'collective', such that when facing severe environmental stress leading to DNA damage and SOS pathway repair, cells trigger genomic island shuffling to produce genetic innovation.

PM171 Comparative genomic analysis provides insight into phylogeny and virulence of atypical enteropathogenic Escherichia coli strains from Brazil

Rodrigo Tavanelli Hernandes[1], Tracy H. Hazen[2], Luís F. dos Santos[3], Taylor S. Richter[2], Jane M. Michalski[2], David A. Rasko[2]

[1]São Paulo State University (UNESP), Department of Microbiology and Immunology - Institute of Biosciences, Botucatu, Brazil
[2]University of Maryland-School of Medicine, Institute for Genome Sciences, Department of Microbiology and Immunology, Baltimore, United States
[3]Instituto Adolfo Lutz, São Paulo, Brazil

Background: Atypical enteropathogenic *Escherichia coli* (aEPEC) is one of the most common diarrheagenic *E. coli* pathotype detected in diarrheal children in Brazil. aEPEC is defined as *E. coli* that harbor the Locus of Enterocyte Effacement, but lack the EAF plasmid and Shiga toxin-encoding genes.

Objectives: The objective of this study was to evaluate the phylogeny and the gene content of aEPEC Brazilian genomes compared to a global collection of aEPEC.

Methods: Single nucleotide polymorphism based phylogenomic analysis was used to compare 328 aEPEC genomes (106 newly sequenced Brazilian genomes and 222 from a global distribution). Large-scale Blast score ratio was used to determine the shared versus unique gene content of the aEPEC genomes.

Results: Phylogenomic analysis revealed the existence of five novel aEPEC phylogenomic lineages, four in phylogroup B1 (designated EPEC11-14), and one in phylogroup A (EPEC15). The 106 Brazilian genomes were assigned in the phylogroups B1 (47.2%), B2 (23.6%), A (22.6%), and E (6.6%); and the majority were classified in the following phylogenomic lineages: EPEC10 (17.9%), EPEC11 (14.2%), EPEC9 (10.4%) and EHEC2 (7.5%). Moreover, we identified 228 genes that were more prevalent (p<0.001) among the Brazilian genomes. Our data support the concept that aEPEC have emerged many times in distinct *E. coli* genetic backgrounds that are identifiable as unique phylogenomic lineages. In addition, the more prevalent genes found among Brazilian genomes could be of great importance to better understand virulence strategies used by these aEPEC to cause disease. Financial support: NIAID, NIH (U19 AI110820) and FAPESP (2015/26207-6 and 2016/17584-3).

PM172 Reversible Gene Expression Control in Yersinia pestis Using an Optimized CRISPRi system

Tong Wang[1], Ruifu Yang[1], Zongmin Du[1]

[1]*Beijing Institute of Microbiology and Epidemiology, Beijing, China*

Background: Many genes in *Yersinia pestis*, the causative agent of three plague pandemics, remain uncharacterized, greatly hampering the development of measures for plague prevention and control. Clustered regularly interspaced short palindromic repeats interference (CRISPRi) has been shown to be an effective tool for gene knockdown. However, the CRISPRi approach in this species is not available yet.

Objectives: Here, we introduce an optimized CRISPRi system using *Streptococcus pyogenes* Cas9-derived dCas9 for gene knockdown in *Y. pestis* to provide a useful tool for Yersinia.

Methods: An aTc-inducible dSpCas9 system successfully used in *E. coli* caused strong gene repression in the absence of inducer when used in *Y. pestis*. We improved the system by introducing P_{L2tetO}, a derivative of the ATc-inducible $P_{LtetO-1}$ promoter, to drive the expression of sgRNA, and by adding TetR to the high copy number sgRNA-expressing plasmid along with the addition of multiple *tetO* elements to the promoter region of *dCas9*.

Results: Ten genes encoded on either the chromosome or plasmids of *Y. pestis* were efficiently knocked down (up to 380-fold) in a strictly anhydrotetracycline-inducible manner using our optimized CRISPRi approach. Knockdown of *hmsH* (responsible for biofilm formation) or *cspB* (encoding a cold shock protein) resulted in greatly decreased biofilm formation or impaired cold-tolerance in *in vitro* phenotypic assays. Furthermore, silencing of virulence-associated genes *yscB* or *ail* using this CRISPRi system resulted in attenuation of virulence in HeLa cells as well as in mice similar to that previously reported for *yscB* and *ail* null mutants.

PM173 De novo transcriptome analysis of Pyropia yezonesis with high-growth mutant using RNA-sequencing

Seo-jeong Park[1], Kim Ngan Tran[1], Jong-il Choi[1]

[1]Chonnam National University, Department of Biotechnology and Bioengineering, Interdisciplinary program for Bioenergy and Biomaterials, Gwangju, Korea, Rep. of South

Background: *Pyropia yezonesis* is one of macro-alga and inhabits in intertidal zones. *P. yezonesis* is used as food in East Asia and its demand is getting increase from many other countries because *Pyropia* is known as healthy food. To meet increased demand, production of *Pyropia* is need to increase and one of the way is development of high-growth *Pyropia* strains. The high-growth mutant strain was developed by gamma radiation.

Objectives: It's difficult understanding the mechanisms of high-growth on the molecular basis due to lack of information. RNA-sequencing is usceful tool for research of non-model organisms like *Pyropia* with transcriptome analysis. This study aimed understanding the mechanisms of high-growth *Pyropia* mutant with transcriptome analysis using RNA-seq.

Methods: RNA extracted from wild-type and mutant *P. yezonesis* and sequenced. The raw data was trimmed to remove adapter sequence and low quality sequences. The clean read data was *de novo* assembled by Trinity. The assemblies were annotated in NCBI NR, Swiss-Prot, Pfam, KEGG, GO and KOG databases.

Results: After sequencing, a total of 167,110,896 paired-end reads with a length of 151 nucleotides, were obtained. *De novo* transcriptome assembly and redundancy removal generated 19,441 transcripts. As a result of transcirptome analysis, we found the activated photosynthesis and nitrogen uptake and assimilation and change of hometostasis in high-growth mutant.

PM174 Analysis of antimicrobial resistance in a community in Malaysia

Jacky Dwiyanto[1], Nazifa Tabassum[1], Kuan Ong[1], Jia Hor[1], Jonathan Levins[1], Muhammad Zoqratt[1;2], Li Wong[1], Jun Khoo[1], Jar Mar[1], Nazmul Hasan[1], Shaun Lee[3], Daniel Reidpath[4], Qasim Ayub[1;2], Sadequr Rahman[1;2], Lee Sui Mae Lee[1]

[1]*Monash University Malaysia, School of Science, Bandar Sunway, Malaysia*
[2]*Monash University Malaysia, Tropical Medicine and Biology Platform*
[3]*Monash University, School of Pharmacy, Malaysia*
[4]*Monash University, School of Medicine, Malaysia*

Background: Antimicrobial resistance (AMR) is of great concern all around the world. However there is limited data on the prevalence of AMR in the community in South East Asia. A well-studied community from Southern Malaysia was sampled to obtain information about the presence of AMR in gut bacteria .

Objectives: The objectives of the study was to determine the prevalence of beta lactam resistance and vancomycin resistance, as appropriate, among Enterobacteriacea, , *Acinetobacter baumannii and Enterococcus faecalis/faecium* (all members of the ESKAPE group of pathogens).

Methods: Dilutions of fecal extracts were cultured and antibiotic resistance profiles of isolates were determined. Bacteria were identified through 16S sequencing. Selected isolates were characterised by multilocus sequence typing (MLST) and amplification and sequencing of diagnostic PCR products.

Results: Fecal samples were collected from 233 people belonging to 110 households from Segamat in Southern Malaysia. Details of the participants are maintained by a health and demographic surveillance system (South East Asia Community Observatory). About 60% of the respondents were found to contain Enterobacteriaceae that exhibited antibiotic resistance. Further analyses of the beta-lactamase resistance revealed that , 25% of the resistance was due to putative ESBL genes and 33% was due to AmpC genes. Antibiotic resistant *Acinetobacter baumannii* and Enterococcus was also isolated from the community and environmental sources. MLST typing demonstrated the presence of multiple clonal lineages in each of the species suggesting that AMR was being spread primarily by plasmid transfer.

PM175 Phage-plasmids: Elements between phages and plasmids

Eugen Pfeifer[1], Eduardo Rocha[1]

[1]*Institut Pasteur, Department of Genomes and Genetics, Microbial Evolutionary Genomics, Paris, France*

Background: Phages and plasmids represent two major classes of mobile genetic elements (MGEs) that are described to be key drivers of bacterial evolution. Although in literature, they are clearly distinguished, several studies reported MGEs that resemble plasmids encoding bona fide phage genes (or vice versa). The most prominent example of these "phage-plasmids" is the phage P1 from *Escherichia coli*, but, in the recent years more and more phages and plasmids were isolated with similar patterns.

Objectives: The distribution and the impact of phage-plasmids are poorly understood. In our work, we focus on the characterization of known phage-plasmids and the identification and analysis of novel elements to understand how they impact bacterial evolution.

Methods: As a first step, we mined plasmid and phage databases for phage-plasmids using prophage-prediction tools, such as PHASTER and VirSorter, and an in-house developed plasmid specific annotation pipeline. Potential candidates are examined by specifically chosen phage- and plasmid specific hidden Markov models of protein sequences, and finally, the hits are evaluated using a random forest analysis.

Results: A database with >400 candidates was set-up with phage-plasmids of Gram-positve and negative bacteria mainly isolated from *Klebsiella, Escherichia* and *Bacillus* species. Currently, we study in detail the genomic content and the genetic organization especially of genes encoding beneficial traits. In conclusion, our work provides first global insights on elements that belong to a novel type of MGE laying between phages and plasmids.

PM176 Gene duplications and virulence in Escherichia coli

Manuel Bernabeu[1], José Francisco Sánchez Herrero[1;2], Pol Huedo[3], Alejandro Prieto[1], Mário Hüttener Queiroz[1], Julio Rozas[1;2], Antonio Juarez[1;4]

[1]*University of Barcelona, Barcelona, Department of Genetics, Microbiology and Statistics,, Spain*
[2]*Biodiversity Research Institute (IRBio), Spain*
[3]*Institute of Biotechnology and Biomedicine (IBB), Spain*
[4] *Institute for Bioengineering of Catalonia (IBEC), Spain*

Background: Bacterial nucleoid associated proteins play relevant roles in the regulation of gene expression. To that group belong the H-NS and Hha proteins. Most enterobacteria encode paralogues of both. In a recent report, we noticed that the genome of the enteroaggregative *E. coli* strain 042 encodes two additional *hha* paralogues, that we termed *hha2* and *hha3*. These paralogues are encoded by virulent *E. coli* isolates that belong to a wide range of pathotypes.

Objectives: To assess whether the presence of additional *hha* paralogues is linked to other genes, hypothetically those that should be targeted by them. Moreover, to investigate the extent of the gene duplications in the 042 genome and their distribution among pathotypes.

Methods: The PancoreTool software was used to compare the genomes of two five-strain sets, one of them including strains that encode a *hha2/hha3* and the other including strains that do not express them. To identify duplications BlastP was used, following the restrictive parameters: >85% similarity, >85% alignment length and e-value < $10e^{-10}$.

Results: Strains encoding *hha2/hha3* also encode the *yeeR aec69 aec70* gene cluster, which maps next to *flu*. This cluster is encoded in a wide variety of *E. coli* isolates and is duplicated in strain 042 itself and several others. Duplications in strain 042 grouped in three main regions. Further analyses have shown that the duplication of region 2 is widespread in strains belonging to most *E. coli* pathotypes. Hence, genes encoded in this region may play a relevant and yet undescribed role in virulence.

PM177 Novel Linear Lipopeptides of Streptomyces sp. KO-7888 Produced by Activation of SARP Regulatory Gene of a Cryptic NRPS Gene Cluster

Wilaiwan Koomsiri[1], Arinthip Thamchaipenet[1], Takuji Nakashima[2], Yuki Inahashi[2], Shiomi Kazuro[2], Yoko Takahashi[2], Satoshi Omura[2], Pakorn Wattana-amorn[1], Markiyan Samborskyy[3], Peter F. Leadley[3]

[1]*Kasetsart University, Genetics, Bangkok, Thailand*
[2]*Kitasato University, Minato City, Japan*
[3]*Department of Biochemistry, Sanger Building, University of Cambridge, United Kingdom*

Background: Actinomycetes are major antibiotic-producing bacteria. However, drug discovery from actinomycetes has been declined due to limitation of laboratory growth condition and bioassay. Recenty, genome analysis from actinomycetes revealed enormous number of uncharacterized biosynthetic gene clusters (BGCs). Several molecular approaches including overexpression of transcriptional regulatory genes such as *Streptomyces* antibiotic regulator protein (SARP) were used to activate corresponding cryptic gene clusters to generate novel compounds.

Objectives: To activate a cryptic non-ribosomal peptide synthetase (NRPS) BGC from *Streptomyces* sp. KO-7888 by overexpression of SARP regulatory gene to discover novel lipopeptides.

Methods: Genome of *Streptomyces* sp. KO-7888 was annotated and predicted for secondary metabolite BGCs. A SARP regulatory gene, *speR*, was identified located adjacent to cryptic NRPS BGC. The *speR* was cloned, then, was intergenerically conjugated to *Streptomyces* sp. KO-7888. The culture of recombinant strain grown in YD medium was extracted and purified. The candidate compounds were identified by LC-UV and structural configurated by the advanced Marfey's method, HR-ESIMS, and NMR.

Results: Genome of *Streptomyces* sp. KO-7888 contained 25 BGCs including cryptic NRPS gene cluster consisting of *speR* gene. Overexpression of *speR* in *Streptomyces* sp. KO-7888 activated corresponding NRPS BGC and generate novel lipopeptides designated sarpeptin A and B. Structure elucidation of sarpeptins revealed 7 amino acids of glycine-glycine-OH-aspartate-L-tyrosine-L-thrionine-D-leucine-OH-aspartate amide connected with fatty acid moieties of 8-methyl-2,4-nonadienoic acid for sarpeptin A and 8-methyl-2,4-decadienoic acid for sarpeptin B. The results indicated that expression of uncharacterized SARP regulatory gene of the cryptic BGC is a productive approach to discover novel compounds from actinomycetes.

PM178 Molecular epidemiology of disease-causing Streptococcus agalactiae from adult patients, 2014-2015

Uzma Basit Khan[1], Elita Jauneikaite[2], Robert Andrews[3], Victoria Chalker[4], Brad Spiller[5]

[1]*Cardiff University, Division of Infection and Immunity, School of Medicine, Cardiff, United Kingdom*
[2]*Imperial College London, Department of Infectious Disease Epidemiology, School of Public Health, United Kingdom*
[3]*Cardiff University, Systems Immunity University Research Institute and Division of Infection and Immunity, School of Medicine, United Kingdom*
[4]*Public Health England, National Infection Service, United Kingdom*
[5]*Cardiff University, Division of Infection and Immunity, School of Medicine, United Kingdom*

Background: *Streptococcus agalactiae* (Group B Streptococcus, GBS) is an important cause of infections in adults with estimated blood infection rates of 3.9/100,000 population for England, Wales and Northern Ireland combined. Capsular serotypes are the main target for GBS vaccine development, however, vaccination may exert a selective pressure for virulent genotypes to switch capsules and escape vaccine coverage.

Objectives: The aim of the current work is to study the molecular epidimeology and determine recombination events leading to potential capsule swtich in adult GBS strains.

Methods: A total of 195 GBS isolates causing invasive and non-invasive infections among adults in England and Wales between January 2014 and August 2015 were serotyped and whole genome sequenced. Genomic analysis determined MLST and presence of virulence factors such as surface protein, *hvgA,* and pilus island genes.

Results: GBS serotype Ia (29.2%), III (29.2%) and V (14.8%) were found to be most prevalent in causing adult diseases. A total of 15.8% (n=31) GBS strains were *hvgA* positive. Five major clonal groups accounted for 94.3% of all isolates clustered in CC1, CC8, CC17, CC19 and CC23. All 195 isolates carried an alpha or alpha-like protein gene, and specific associations between genes and serotypes, such as serotype Ib and II/*bca*, serotype III/*rib*, and serotype V/*alp3*, reflected the presence of specific genetic lineages. Multiple recombination events leading to potential capsular switch in two serotype Ib and II /ST1 isolates each from serotype V and one serotype IV/ CC17 lineage from serotype III were observed.

PM179 In vitro bactericidal and time-kill profile of ethyl acetate crude extract of psammopsinia sp collected from algoa bay, south africa

Anthony Otigbu[1], curtbert kibungu[2]

[1]*university of fort hare, Biochemistry and Microbiology, Alice, South Africa*
[2]*University of Fort Hare, Biochemistry and Microbiology, Alice, South Africa*

Background: Prevalence of drug resistance among bacterial species has been primarily attributed to chromosomal encoded-resistance, Qnr proteins (aminoglycoside acetyltransferase AAC (6')-Ib-cr) and high expressions of active efflux pump systems (*QepA* and *OqxAB*), resulting in assemblage of mutations. These pentapeptide repeat amino acids are suggested to be origins of aquatic environments.

Objectives: This study aims to investigate the kill-time inhibitory potentials of active compound extracts from *Psammopsinia* sp (a marine sponge) on selected waterborne pathogens with determined antimicrobial profile isolated from river/estuary water.

Methods: Sponge species was collected from Phillip's Reef in Algoa Bay, Port Elizabeth, South Africa in spring and extraction of compounds was performed using ethyl acetate following specimen identification. A 100%, 75% and 50% v/v concentration of the extract was used to perform the kill-time activity against confirmed pathogens at time intervals of 0, 4 and 24 hours. Absorbance readings were measured at 600 nm. The bioactive compounds present in *Psammopsinia* sp crude extracts were identified by HPLC-MS. and GC-MS

Results: Most significant bioactivity (0.003-0.009) was observed at 0 hr interval on 46.2% of test isolates with 50% v/v concentration, 38.5% isolates with 100% v/v concentration and 23.1% with 75% v/v concentration. No activity was observed at against all test isolates at 24 hr interval with 75% v/v concentration. *Enterococcus faecalis* was the most inhibited with 100% and 50% v/v concentration at all intervals while least effect was recorded against *Campylobacter jejuni* (0.524 and 0.568) at 24 hr interval.

PM180 Rapid detection of antibiotic resistant escherichia coli in blood using flow cytometry

Luminita Marutescu[1;2], Alexandra Velican[1;2], Otilia Banu[3], Elvira Borcan[4], Marcela Popa[1], Carmen Chifiriuc[1;2]

[1]*Research Institute of the University of Bucharest – ICUB, Microbiology Department, Bucharest, Romania*
[2]*University of Bucharest, Faculty of Biology, Microbiology University Department, Bucharest, Romania*
[3]*Institute of Cardiovascular Diseases Prof. C.C. Iliescu, Bucharest, Romania*
[4]*Clinical Institute Fundeni, Bucharest, Romania*

Background: *Escherichia coli* is the etiological agent of 17–37% of bloodstream infections and theemergence of resistance in these strains urged the exploration of rapid detection methods for guiding the emergency antibiotic treatment.

Objectives: to develop a rapid analysis protocol based on flow cytometry (FC), able to provide early information about of the presence/resistance of *E. coli* in blood, within 2 hours.

Methods: Standard antimicrobial susceptibility testing (AST) was performed with Vitek-2 and broth microdilution. The FC-based AST was performed on blood specimens deliberately contaminated with *E. coli* strains (n=30). Bacterial suspensions prepared from mid logarithmic liquid cultures were inoculated in freshly anticoagulated human blood containing five different antibiotics (amoxicillin+clavulanic acid, cefotaxime, meropenem, ciprofloxacin, amikacin) to a final density of 5 x 10^5CFU/mL. After lysis, samples were individually stained with propidium iodide (PI) and carboxyfluoresceinsuccinimidyl ester (CFSE) viability markers. The intensity of fluorencence (IF) wasmeasured with an Accuri C6 plus flow cytometer and analyzed with the FlowJo software

Results: Forward scatter and the ratio of green to red fluorescence proved to discriminate *E. coli* from the remaining eukaryotic cells and debris. The FC assays results showed a good correlation with those of standard AST methods. Susceptible isolates showed an increased red fluorescence after antibiotic treatment. The FC method could provide relevant data regarding the presence of *E. coli* cells in blood and their resistance phenotypes, much earlier than conventional methods. This preliminary information could reduce the selective pressure and exposure to inappropriate therapy.

PM181 Anti-pathogenic effect of Eugenia caryophyllata essential oil functionalized core-shell magnetic nanoparticles

Alina Maria Holban[1;2], Alexandru Mihai Grumezescu[2], Carmen Curutiu[3], Lia-Mara Ditu[3], Irina Gheorghe[3], Bogdan Vasile[2], Coralia Bleotu[4], Veronica Lazar[3], Ecaterina Andronescu[2]

[1]*University of Bucharest, Department of Microbiology and Immunology, București, Romania*
[2]*Politehnica University of Bucharest, București, Romania*
[3]*University of Bucharest, București, Romania*
[4]*Ştefan S. Nicolau Institute of Virology*

Background: As the phenomenon of antimicrobial resistance is constantly expanding, novel solutions are urgently nedeed to combat severe infections. Nanosystems are curently investigated to increase the specificity and efficiency of antimicrobials by allowing the use of lower amounts of drugs and modulation of microbial behaviours controlling the infectious process.

Objectives: This study aimed to obtain and characterize core-shell nanoparticles (NPs) based on *Eugenia caryophyllata* essential oil (EO) functionalized magnetite, able to modulate virulence and pathogenicity of clinically relevant microbial species.

Methods: Functional NPs were obtained by co-precipitation and physico-chemically characterized by TEM, SEM, IR and XRD. Laboratory and clinical isolates belonging to *Pseudomonas aeruginosa, Staphylococcus aureus* and *Candida albicans* species were used to investigate the impact of the obtained NPs on microbial growth, attachment, biofilm formation and soluble virulence factors production.

Results: The study revealed that EO functionalized core-shell NPs present different minimum inhibitory concentrations (MICs) ranging from 0,06mg/mL (*C.albican*s) to 0,5 mg/mL (for *P. aeruginosa* clinical isolates) in planktonic cultures while biofilm formation is inhibited by concentration higher than 0,125 mg/mL in all strains. Subinhibitory concentrations of the obtained aqueous NPs dispersions significantly inhibited microbial attachment both on the inert and cellular substrata (HeLa cultures) of the tested strains and modulated the production of soluble virulence factors in a strain and concentration manner. MIC/2 concentrations have the most significant inhibitory effect in *C. albicans* filamentation and also inhibition of pore toxin enzymes in *S. aureus* and *P. aeruginosa*, demonstrating that the obtained NPs may have significant antipathogenic effects.

PM182 Comparative genomic analysis reveals high intra-serovar plasticity within Salmonella Napoli isolated in Europe, 2005-2017

Eleonora Mastrorilli[1;2], Massimiliano Orsini[3], Sara Petrin[1;2], ALESSANDRA LONGO[1;2], Debora Cozza[4], Ida Luzzi[5], Antonia Ricci[6], Lisa Barco[1], Carmen Losasso[1;2]

[1]*Istituto Zooprofilattico Sperimentale delle Venezie, National reference centre for salmonellosis, Legnaro, Italy*
[2]*Istituto Zooprofilattico Sperimentale delle Venezie, Microbial Ecology Unit, Legnaro, Italy*
[3]*Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale", Bioinformatics, Teramo, Italy*
[4]*Istituto Zooprofilattico Sperimentale del Mezzogiorno, Food Inspection, Portici, Italy*
[5]*Istituto Superiore di Sanità, DMIPI, Roma ,*
[6]*Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Italy*

Background: Differently from other European countries, *Salmonella* Napoli is among the top serovars causing human infections in Italy; it is mainly isolated from humans and environment, but neither the reservoir nor its route of infection are known. This serovar is characterized by high genomic diversity and epidemiological, clinical and molecular evidences revealed important similarities with typhoidal *Salmonella* serovars.

Objectives: To investigate the epidemiology and pathogenicity of *S*. Napoli using comparative genomic analysis. Collected data could be valuable to identify putative vehicles of human infection and assess control measures.

Methods: 140 *S*. Napoli Italian isolates were collected and sequenced and 38 European *S*. Napoli sequences were obtained from ENGAGE consortium (http://www.engage-europe.eu/), all spanning years 2005-2017. Moreover, all *S*. Napoli, Typhi, Paratyphi A, Choleraesuis, Newport, Enteritidis, Dublin, Heidelberg, Typhimurium and 1,4,[5],12:i:- reference genomes available on GenBank were retrieved. Bioinformatic analysis included assembly, annotation, core genome tree building and accessory genome Gene Ontology enrichment. Samples were also characterized in terms of Sequence Type (ST), plasmid replicons, Salmonella Pathogenicity Islands, antimicrobial resistance genes, biocides and metal resistance genes.

Results: Preliminary results confirm high genetic variability of this serovar, with more phylogenetic substructure than previously known. Few *S*. Napoli samples show horizontally acquired determinants. *S*. Napoli samples tend to cluster according primarily to ST, with patterns of gene insertion/deletion characterizing each ST. Notably, ST-474, the oldest of our collection, is biphyletic. Moreover, all STs spread among isolation sources and years of isolation, highlighting the challenge this serovar poses to trace its epidemiology and evolution.

PM183 Evaluation of three methodologies for the in vitro susceptibility testing of Ceftolozane-Tazobactam (C/T)

Jonathan Mark Tyrrell[1], Emma Widlake[1], Timothy Rutland Walsh[1]

[1]*Cardiff University, Department of Medical Microbiology & Infectious Disease, Division of Infection & Immunity, School of Medicine, United Kingdom*

Background: Since 2014, Ceftolozane-Tazobactam (C/T) has been an effective therapeutic β-lactam/β-lactamase inhibitor combination against multidrug-resistant (MDR) Gram-negative pathogens, particularly within the clinical indications of complicated urinary tract infections (cUTI) and intra-abdominal infections (cIAI). Accurate deduction of C/T MICs is imperative to its clinical stewardship.

Objectives: To evaluate and compare the MIC testing of C/T by 3 methods, (i) broth microdilution (BMD), used as a reference method (ii) MIC Test strips (MTS) (Liofilchem®), (iii) the BMD-based ComASP Ceftolozane-Tazobactam (Liofilchem®).

Methods: A random sample of clinical *Enterobacteriaceae* (n=160) were selected, including MDR *Escherichia coli* and *Klebsiella pneumoniae*. C/T MICs were determined by each of the aforementioned methods. Each strain underwent all 3 methods within the same day of testing. After typical overnight incubation, MICs were read and interpreted as defined by EUCAST. Essential agreement (EA) was classified where C/T MICs were plus/minus 1x 2-log dilution of those read for the BMD reference method. Categorical agreement (CA) was defined as the MICs that did not change the categorisation of isolates as susceptible/intermediate/resistant.

Results: The ComASP C/T panel showed significant levels of EA (95.6%) and CA (92.5%) when compared to the reference method (BMD). In contrast, the C/T MTS performed marginally worse in comparison, with EA and CA of 92.5% and 92.5% respectively. This current pilot data suggest that ComASP C/T may represent a viable alternative to more complex and laborious BMD. Further data collection is currently being undertaken to increase the significance and reliability of our conclusions.

PM184 Comparison of molecular detection methods of human pathogens using proficiency testing.

Bernard China[1], Kris Vernelen[1]

[1]*Sciensano, Quality of laboratories, Brussels, Belgium*

Background: Since 2008, the Belgian medical laboratories willing benefit to the of their tests in molecular microbiology must be accredited under ISO15189 standard. Among the requirements, those laboratories must participate to External Quality assessment. Sciensano is in charge of the organization of proficiency testing for the medical laboratories in Belgium.

Objectives: Since 2008, we organize the proficiency testing for the medical laboratories for the molecular detection and/or quantification of some human pathogens. The aim is to evaluate the proficiency of the Belgian laboratories per pathogen and per method. In order to identify the best diagnostic method for the detection of a specific pathogen in a specific matrix.

Methods: The studied parameters are: C. trachomatis, N. gonorrhoeae, M. tuberculosis, T. gondii, enteroviruses, VZV, HSV1, HBV, HCV and HCV genotyping. The EQA panels were self-produced or provided by QCMD (Scotland). Each panel contained positive and negative samples. Each participating laboratory received a panel, performed the molecular detection and encoded their results on line. For each result the laboratory receives a score: 0 for a perfect result, 1 for a wrong result for an infrequently detected sample, 2 for a frequently detected sample and 3 for a frequently detected sample or for a false positive result. The results were evaluated per sample, per method and per laboratory.

Results: The EQA allows us to evaluate the proficiency of the laboratories and the used methods. An improvement was observed with time indicating that both the laboratories and the companies improved their methods and their kits.

PM185 Impact of epinephrine on the adherence and biofilm formation of Pseudomonas aeruginosa

Mélyssa Cambronel[1], Sophie Rodrigues[1], Julie Hardouin[2], Olivier Maillot[1], Marc Feuilloley[1], Nathalie Connil[1]

[1]*Laboratory of Microbiology Signals and Microenvironment - LMSM EA 4312, Évreux, France*
[2]*Laboratoire Polymères, Biopolymères, Surfaces Equipe BRICS, MONT SAINT AIGNAN , France*

Background: Microbial endocrinology is, nowadays, a well-known field, highlighted by Mark Lyte in the nineties. He showed that eukaryotic molecules were able to modulate the physiological behavior of the bacteria inside the human body. Since then, a lot of studies have shown that various substances can act on bacteria as hormones, neurotransmitters, and molecules of the immune system. Among them, the hormones/neurotransmitters, epinephrine and norepinephrine, released in case of stress, physical efforts or used in medical treatment, were shown to be able to modify the mobility behavior as well as bacteria's capacity to form biofilm in various species and mostly in *Escherichia coli*.

Objectives: To study the effect of epinephrine on the physiology of *Pseudomonas aeruginosa*, a bacteria unfortunately famous to be linked to a lot of hospital-acquired infections, and responsible of chronic infection in immunocompromised patients including patients suffering from cystic fibrosis.

Methods: In our study, we investigated the physiological response of *P.aeruginosa* towards epinephrine through different motility assays (swarming, swimming and twitching), and by observation of adherence on abiotic surface in static conditions as well as in dynamic ones. Confocal microscopy was notably used to monitor the impact of this hormone on biofilm formation.

Results: In this work, *P. aeruginosa* treated with the hormone epinephrine seems to increase its adherence capacity in static conditions as well as in dynamic conditions. It is also able to modulate its biofilm formation.

PM186 Transient colonization of MRSA on the stratum corneum of human skin is controlled by a local IL-8 mediated neutrophil recruitment

Anette Schulz[1], Long Liang[2], Lisanne de Vor[3], Markus Ehrström[4], Fredrik Wermeling[2], Liv Eidsmo[2], Keira Melican[1]

[1]*Karolinska Institut, Neuroscience, Stockholm, Sweden*
[2]*Karolinska Institut, Center for Molecular Medicine, Stockholm, Sweden*
[3]*University Medical Center Utrecht, Utrecht, Netherlands*
[4]*Karolinska Institut, Department of Medicine, Stockholm, Sweden*

Background: Epi-cutaneous *Staphylococcal* colonization is increasingly recognized as important for priming the host dermal immune response towards this potential pathogen.

Objectives: The significant morphological and immunological differences between human and mouse skin encouraged us to develop a xenograft humanized model in which we study *S. aureus* skin colonization on living human skin.

Methods: In our *in vivo* human skin xenograft mouse model, *S. aureus* was topically applied to human skin. After 2, 5 and 10 days, samples were prepared for viability counts, cytokine analysis (Luminex), immunohistochemistry and flow cytometry (day 5). To study the influence of neutrophils in colonisation, we performed transmigration assays using explant tissues homogenates and induced neutropenia *in vivo* before inoculation of human skin with bacteria.

Results: We have identified an IL-8 mediated pro-inflammatory signalling that is upregulated in response to epi-cutaneous colonization of human skin by *S. aureus* USA300 *in vivo* and *in vitro*. The IL-8 response appears to be produced by the surface layers of keratinocytes and does not require invasive bacteria. This pro-inflammatory signal induces directed transmigration of neutrophils into the thick human epidermis. Blockage of this IL-8 signal *in vitro* is shown to reduce neutrophil transmigration. Neutrophil depletion *in vivo* leads to higher bacterial loads on the skin indicating that this epidermal neutrophil recruitment may function to control bacterial numbers on the skin surface. This work has demonstrated a unique human response to epi-cutaneous *Staphylococcus* and we hypothesise that this sub-clinical response is a way for the tissue to control bacterial numbers.

PM187 Characterization of traveler's diarrhea outbreak-associated campylobacter jejuni in china

Mei Qu[1], Meng Chen[1], Xinghuo Pang[1]

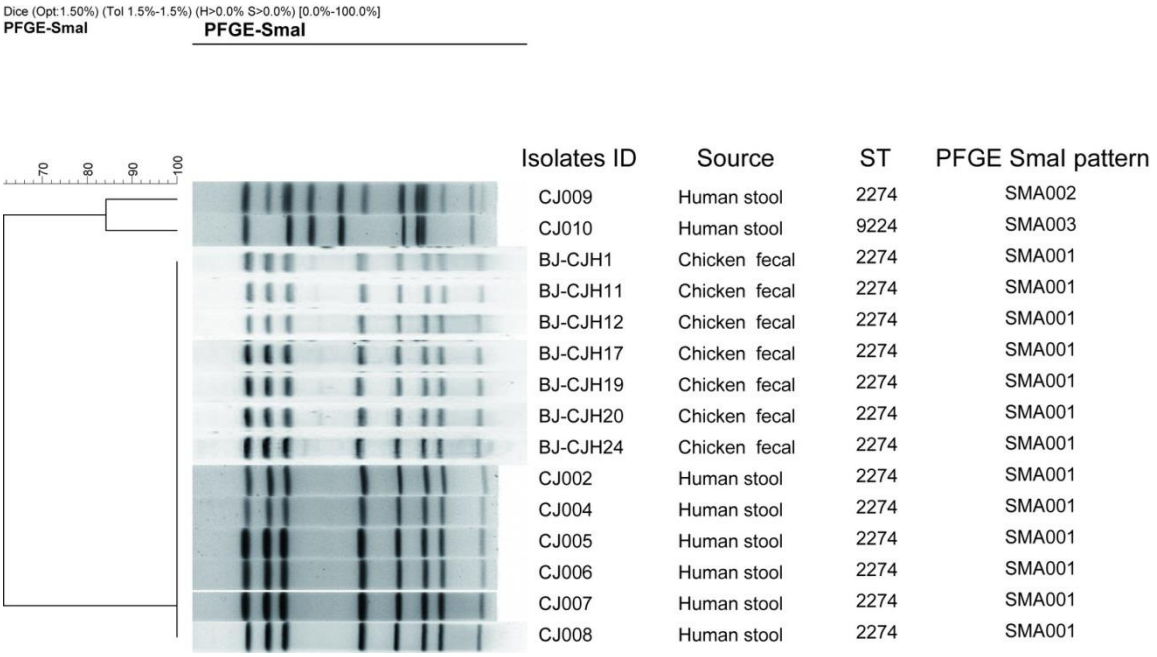
[1]*Beijing Center for Disease Prevention and Control, Beijing, China*

Background: One 36 cases of acute gastroenteritis occurred in a high school in Beijing, during a trip to other province in April 2018.

Objectives: The epidemiological investigation and laboratory detection for the enteric pathogen infection was conducted to identify the cause.

Methods: Eighteen stool specimens from 11 diarrheal patients and 3 close contacts were collected and tested for 16 enteric bacterial and viral pathogens using real-time PCR methods. Multilocus Sequence Typing (MLST), pulsed-field gel electrophoresis (PFGE) and antimicrobial susceptibility testing were applied in this outbreak in order to characterize the incident.

Results: According to the distribution of time, the number of onset people with rapid rise and decline in a short period of time, a single peak curve showed that it was a traveler's diarrhea outbreak. Ten (90.9%) of 11 stool specimens from diarrheal patients were positive for *Campylobacter jejuni*, confirmed as the cause of outbreak. Eight *C. jejuni* isolates were obtained and produced 2 sequence types and 3 PFGE patterns. Six isolates had the same ST and PFGE pattern which indicated this outbreak was initiated by a mixed *C. jejuni* infection. All 8 isolates were multi-drug resistant, with the dominant resistance pattern of nalidixic acid, tetracycline and ciprofloxacin combined, except for 2 isolates resistant to florfenicol. This was the first recognized acute gastroenteritis caused by *C. jejuni* among 20 years in China. This investigation emphasizes the significance of strengthening Campylobacter's active surveillance and identification of the outbreak source in future.



PM188 Evaluation of constitutively type 1 fimbriated and nonfimbriated Salmonella Typhimurium strains as vaccine strains

Kuang-Sheng Yeh[1], Yi-Hsuan Huang[2], Hsing-Jung Wu[1]

[1]National Taiwan University, Veterinary Medicine, Taipei, Taiwan
[1]National Taiwan University, Veterinary Medicine, Taipei, Taiwan

Background: Type 1 fimbriae is the most common fimbrial type in *Salmonella* Typhimurium. The *fim* gene cluster is responsible for the expression of type 1 fimbriae. Our studies have established several *S*. Typhimurium strains with *fim* regulatory gene deletions. The *fimZ* and *fimY* mutants were nonfimbriated, whereas the *stm0551* and *fimW* mutants constitutively expressed type 1 fimbriae.

Objectives: Most *Salmonella* live vaccines are constructed by deleting virulence genes to attenuate *Salmonella*. Another approach, attenuating gene expression, attenuates microorganisms through overexpression of surface appendages. We aimed to compare the virulence of the constitutively type 1 fimbriated and nonfimbriated *S*. Typhimurium strains to evaluate whether they could serve as vaccine strains.

Methods: Growth rate, susceptibility to H_2O_2 and bile salt, and membrane stability tests using erythromycin were employed to assess the aforementioned strains.

Results: The *fimY* mutant exhibited a decreased growth rate compared with other strains. The *fimW* mutant was most susceptible to H_2O_2 treatment, whereas the *fimZ* mutant had the lowest survival rate in the medium containing bile salt. The *fimW* and *stm0551* mutants both exhibited lower minimum inhibitory concentration of erythromycin, indicating that their membranes were less stable than those of the parental strains. Our initial assessment demonstrated that these mutants were all somewhat less virulent than the parental strains, warranting further *in vivo* investigation.

PM189 Salmonella Typhimurium Subtle-Invasion of gut epithelial cells

Desiree Boeck[1], Stefan Fattinger[2;3], Maria Letizia Di Martino[3], Sabrina Deuring[2], Markus Furter[2], Saskia Kreibich[2], Francesco Bosia[1], Anna A. Müller[2], Bidong D. Nguyen[2], Manfred Rohde[4], Martin Pilhofer[1], Wolf-Dietrich Hardt[2], Mikael E. Sellin[2;3]

[1]*Institute of Molecular Biology & Biophysics, Department of Biology, ETH Zurich, Zurich, Switzerland*
[2]*Institute of Microbiology, Department of Biology, ETH Zurich, Zurich, Switzerland*
[3]*Uppsala University, Department of Medical Biochemistry and Microbiology, Uppsala, Sweden*
[4]*Central Facility for Microscopy, Helmholtz Centre for Infection Research, Braunschweig, Germany*

Background: *Salmonella* Typhimurium (*S*.Tm) infections of cultured cell lines have given rise to the ruffle model for epithelial cell invasion. According to this model, the type-three-secretion-system-1 effectors SopB, SopE, and SopE2 drive an actin nucleation cascade, resulting in cooperative *S*.Tm uptake. However, cell line experiments poorly recapitulate the cell and tissue features encountered in the host's gut mucosa.

Objectives: We therefore set out to elucidate to what extent current working models suffice to explain bacteria-host cell interplay in the intact intestinal mucosa.

Methods: Here, we have combined imaging and bacterial genetics to compare *S*.Tm invasion of epithelial cell lines and the gut epithelium in mice.

Results: We find that epithelial entry *in vivo* occurs through "subtle-invasion", a process distinct from the previously established model of *S*.Tm invasion. Subtle-invasion requires the conserved *S*.Tm effector SipA, is facilitated by a docking step that involves the SPI-4-encoded *Salmonella* adhesin system, preferentially targets apicolateral hot spots at cell-cell junctions, and shows dependence on local cell neighborhood. Our results highlight the differences between *S*.Tm invasion of cultured epithelial cell lines and the intact intestinal mucosa and provide a mechanistic model for *S*.Tm invasion of the gut epithelium.

PM190 Comparative analysis of two Chlamydia trachomatis strains of human and sheep origin: possible emergence of a novel variant

Valentina Feodorova[1], Sergey Zaitsev[1], Yury Saltykov[1], Edgar Sultanakhmedov[2], Andrew Bakulev[3], Sergey Ulyanov[4], Vladimir Motin[5]

[1]Federal Research Center for Virology and Microbiology, Branch in Saratov, Laboratory for Molecular Biology and NanoBiotechnology, Saratov, Russian Federation
[2]Saratov State Medical University , Department for Skin Diseases, Saratov, Russian Federation
[2]Saratov State Medical University , Department for Skin Diseases, Saratov, Russian Federation
[4]Saratov State National Research University , Department for Medical Physics, Saratov, Russian Federation
[5]University of Texas Medical Branch, Department of Pathology, Department of Microbiology & Immunology, Galveston, TX, United States

Background: *Chlamydia* is globally recognized as both human and livestock pathogen causing a severe symptomatic, as well as asymptomatic chlamydiosis. The recent emergence of novel deletion variants of *Chlamydia* (nvCT) resulted in a new wave of chlamydial outbreaks worldwide. In contrast to the numerous data on nvCT of human origin, our knowledge on nvCT from livestock source is limited to a few reports.

Objectives: The aim of this stidy was to compare the main chromosomal and cryptic plasmid target regions of two *Chlamydia* strains derived from asymptomatic Chlamydia patient with fatal infertility and aborted sheep with confirmed asymptomatic chlamydiosis with detectable level of chlamydial antibodies.

Methods: Clinical specimens from either sick patient or aborted sheep were carefully tested by PCR with relevant primers followed by target sequencing of PCR fragments, as well as PCR testing for the presence of DNA of other known pathogens with abortogenic potential.

Results: We found no specific DNAs for any known abortogenic pathogens except nvCT DNA in clinical specimens derived from human and sheep. Both human and sheep nvCT variants belonged to the genovar E, and showed the presence of a 377 bp deletion in the plasmid *orf1* gene, a duplication of 44 bp in the plasmid *orf3*, and SNP in *orf4* typically present in human nvCT. In contrast to nvCT, both human and sheep variants contained an additional deletion of 17 bp in *orf1* gene (ddCT), and belonged to MLST sequence type ST13 rather than to ST4 type of typical nvCT. Grant RSF-No.-17-16-01099.

PM191 Incidence of urinary tract infections (UTIs) amongst outpatients attending Primary Health Centres in Anambra State, Nigeria

Chioma Ogbukagu[1], Ikechukwu Ekwealor[1]

[1]*Nnamdi Azikiwe University, Applied Microbiology and Brewing, Awka, Nigeria*

Background: Urinary tract infections (UTIs) are one of the most prevalent extra-intestinal bacterial infections. It is reportedly common among people of all ages. These infections are on the increase for outpatients attending Primary Health Centres in Anambra State, Nigeria, therefore the need for this study.

Objectives: The objectives are: to isolate the pathogens associated with UTI, characterize them and further check their antimicrobial susceptibility patterns.

Methods: Clean-catch midstream urine samples were collected and examined. The organisms were isolated and identified. The effects of gender and age distribution on the prevalence rate of UTI were monitored. Standard broth dilution technique was deployed for monitoring antibiotic susceptibility pattern.

Results: Out of 3000 urine samples examined, 528 bacterial isolates were recovered and characterized. These include: *Escherichia coli* (24.2%), *Klebsiella* sp. (18.2%), *Staphylococcus aureus* (18.2%), *Proteus mirabilis* (9.1%), *Pseudomonas aeruginosa* (9.1%), *Enterococcus faecalis* (9.1%), *Citrobacter intermedius* (6.1%) and *Staphylococcus saprophyticus* (6.1%). More females (52%) were infected than males (48%) and in both sexes, the highest incidence was found amongst the age group, 26–38 years. Gram negative rods had the highest incidence in both sexes. Susceptibility tests indicated that *Staphylococcus aureus* was sensitive to Cephalexin, Penicillin V, Erythromycin and Gentamycin while *Pseudomonas aeruginosa* was resistant to all the antibiotics. *Escherichia coli* and *Klebsiella* sp. were resistant to all the antibiotics except Gentamycin while *Citrobacter intermedius* was resistant only to Cephalexin and Erythromycin. This study provides an evidence of urinary tract infections amongst outpatients and the possible drugs for their control.

PM192 The repertoire of toxin-antitoxin systems in adhesive-invasive E. coli is transcriptionally active in vitro under intestinal- and intramacrophage-like stress conditions. New insight associated to this emergent E. coli pathotype

Paula Bustamante[1], Roberto Vidal[1]

[1]*Programa de Microbiología y Micología, Instituto de Ciencias Biomédicas, Facultad de Medicina, Universidad de Chile, Chile*

Background: Crohn's disease (CD) is a chronic gastrointestinal inflammation as consequence of interactions between an altered resident microbiota and the host innate immune system, in genetically susceptible individuals. Adherent-invasive *Escherichia coli* (AIEC), characterized by its abilities to adhere/invade epithelial cells, and to survive/replicate within macrophages, has been observed increased in CD patients. However, bacterial factors involved in AIEC persistence are currently unknown. In this sense, bacterial toxin-antitoxin (TA) systems, small genetic elements diverse in sequence, antitoxin nature and mechanistic, are recognized locus that participate in persistence and stabilization of mobile genetic elements.

Objectives: Our aim was to define the AIEC TA repertoire, and to analyse *in vitro* the expression of TA toxin genes in response to stress conditions.

Methods: The TA repertoire of the reference AIEC strain NRG857c was explored using an *in house* database; a comparison of their genetic contexts was done using bioinformatic tools. Relative expression levels of toxin genes were evaluated by realtime PCR.

Results: 27 TAs were found in NRG857c, some of them absent or located in different genomic contexts in non-AIEC isolates. TAs harbored by mobile genetic elements encoding virulence factors were also identified. When AIEC strains were culture in presence of bile salts, there was a heterogeneous toxin expression pattern; however, when culture at pH 4.5, roughly all toxin genes were up-regulated. Differences strain dependent were also found.

We hypothesize that TA systems could contribute to AIEC pathogenicity by maintenance of virulence genes encoded in mobile elements, and hence to contribute to AIEC persistence.

PM193 Inertial and acoustic microfluidic platforms for pathogen sample purification

Pablo Rodriguez[1], Charlotte Dyer[1], Alexander Iles[1], Nicole Pamme[1]

[1]*University of Hull, United Kingdom*

Background: Pathogen analysis is often hampered by low concentrations in complex matrices and lengthy cell culturing. Here, we explore microfluidic approaches for label-free continuous-flow focussing to purify pathogens. (1) Inertial microfluidics focusses particles into narrow streams within winding channels due to a balance of inertial lift and Dean drag forces (PNAS, 2007, 104, 18892) depending on curvature, flow rate and size. (2) Acoustofluidics employs a standing wave across the channel to focus microparticles to the node, depending on size, density and compressibility (Analyst, 2004, 129, 938).

Objectives: Investigate the focussing of microparticles, cell models, with inertial and acoustic microfluidics.

Methods: Polystyrene beads (2 µm, 10 µm) or blood were pumped through the microchannels: for inertial focussing, a serpentine channel (175-300 µm wide and 33 µm deep) was employed; for acoustofluidics, a piezoceramic element (2.4 MHz) was utilised in combination with a straight channel (590-305 µm top-bottom wide and 100 µm deep).

Results: Preliminary results showed inertial focussing near the channel edges and isolation of 10 µm beads (98.1%) as well as blood cells (81.7%) at a flow rate of 700 µL min^{-1}. The 2 µm beads were focussed to a lesser extent, indicating that inertial microfluidics may be suitable for initial focussing of larger cells to isolate pathogens followed by downstream pathogen focussing for pre-concentration. With acoustofluidics, 10 µm beads and blood cells were focussed in the centre; however, the focussing of 2 µm particles was not achieved and would thus only be applicable for pre-analytical sample preparation.

PM194 Effects of baicalein on the cytotoxicity, production and secretion of Shiga toxins of enterohaemorrhagic Escherichia coli

Pham Thi Vinh[1], Yui Shinohara[1], Akifumi Yamada[1], Motokazu Naykayama[2], Tadahiro Ozawa[3], Jun Sato[2], Yoshimitsu Masuda[1], Ken-ichi Honjoh[1], Takahisa Miyamoto[1]

[1]Kyushu University, Bioresource and Bioenviromental Sciences, Fukuoka, Japan
[2]Kao Corporation, Global R &D-Safty Science, Japan
[3]Kao Corporation, Bioscience Research, Japan

Background: *Escherichia coli* O157:H7 is an important foodborne pathogen. Polyphenols are increasingly considered as potential antibacterial agents to control foodborne pathogens.

Objectives: The aim of this study is to examine the effects of baicalein on the cytotoxicity of Shiga toxins 1 and 2 (Stx1 and Stx2).

Methods: The Stxs preparation extracted from culture supernatant of *E.coli* was preincubated with baicalein or epigallocatechin gallate (EGCg) before adding to the Vero cells culture. To examine protective effects of polyphenols on Vero cells against Stxs, Vero cells were pretreated with baicalein or EGCg and then Stxs preparation was added to the cells. Titer of Stxs was determine by RPLA assay. Cell viability was determined by using MTT assay. Transcription of *stx* genes was measured by real-time PCR. Docking simulation was performed to examine a possible mechanism for inhibitory action of baicalein against Stxs.

Results: Baicalein inhibited the cytotoxicity of both Stx1 and Stx2 against Vero cells, while EGCg only inhibited Stx1, not Stx2. Baicalein protected Vero cells from the cytotoxicity of both Stx1 and Stx2. In contrast, protective effects of EGCg were not observed on Vero cells against both Stx1 and Stx2. Both baicalein and EGCg increased transcription of *stx1* but not of *stx2*. Baicalein had no effects on the secretion of both extracellular and intracellular Stxs production, while EGCg inhibited the secretion of extracellular Stxs. Docking models suggested that baicalein could stably formed complex with StxB pentamer with low intramolecular energy.

PM195 Unraveling the survival mechanism of P. salmonis in Atlantic salmon macrophages

Diego Pérez-Stuardo[1], Allison Espinoza[1], Valentina Ibaceta[1], Bernardo Brianson[1], Diego Ahumada[1], Jorge Valdés[1], Felipe Reyes-Lopez[2], Elena A. Vidal[1;3], Sebastian Reyes-Cerpa[1]

[1]*Universidad Mayor, Centro de Genómica y Bioinformática, Facultad de Ciencias, Huechuraba, Chile*
[2]*Universitat Autònoma de Barcelona, Department of Cell Biology, Physiology and Immunology, Spain*
[3]*Universidad Mayor, Instituto Milenio de Biología Integrativa iBio, Chile*

Background: *Piscirickettsia salmonis* is the most important health problem on Chilean aquaculture industry. However, the biology of its infective cycle is poorly known. Recently, it has been suggested that *P. salmonis* infects and replicates within macrophages avoiding its lysosomal degradation. However, the interference mechanisms on macrophages immune response to ensure its survival are poorly understood.

Objectives: Analyze the transcriptomic profile and evaluate the functionality of lysosomes of Atlantic salmon macrophages infected by *P. salmonis*.

Methods: The transcriptomic profile of Atlantic salmon macrophages infected by *P. salmonis* was obtained by a microarray analysis using the Salmon Immunity and Quality 15k custom array platform (GPL16555). The lysosome pH and the proteolytic activity of macrophages infected by *P. salmonis* was analyzed using the LysoSensor Yellow/Blue™ probe and the DQ™-BSA-Green probe respectively by confocal microscopy. The relationship between lysosomal activation and *P. salmonis* replication was determined by a gentamicin protection assay.

Results: The transcriptomic profile obtained from macrophages infected by *P. salmonis* shows changes on expression of genes related to the endocytic pathway. When we analyze the lysosomal response of infected cells, we observed that pH was maintained neutral and presents low proteolytic activity, similar as the obtained in non-infected macrophages. Interestingly, *P. salmonis* survive and replicate within Atlantic salmon macrophages. Altogether, the results showed that the infection of *P. salmonis* induce a limited lysosomal response which may could be associated to host immune evasion mechanisms.

PM196 Bacterial load in different tissues of signal crayfish Pacifastacus leniusculus (Dana, 1852) and its potential role as fitness indicator

Paula Dragičević[1], Ana Bielen[2], Ines Petrić[3], Dora Pavić[2], Ivana Maguire[1], Sandra Hudina[1]

[1]Faculty of Science, University of Zagreb, Department of Biology, Zagreb, Croatia
[2]Faculty of Food Technology and Biotechnology, University of Zagreb, Department of Biochemical Engineering, Zagreb, Croatia
[3]Ruđer Bošković Institute, Division for Marine and Environmental Research, Zagreb, Croatia

Background: Invasive non-indigenous crayfish species (NICS) negatively impact ecosystems and native crayfish populations by, among others, introducing pathogens into the new environment. Literature suggests that hemolymph of apparently healthy crayfish is unsterile and can, in stressful conditions, contain a higher bacterial load which may be correlated with higher bacterial load in hepatopancreas. Understanding how bacterial load affects crayfish immunity and fitness would help us assess whether the healthiest individuals are the first to disperse to new environment.

Objectives: To determine whether bacterial load in hemolymph could be used as crayfish fitness proxy and to establish whether there is a link between bacterial load in hemolymph and hepatopancreas of the most successful crayfish invader in Europe – the signal crayfish (*Pacifastacus leniusuclus*), in the Korana River, Croatia.

Methods: We collected over 110 individuals along the Korana River and sampled cuticule swabs, and hepatopancreas, gut and hemolymph tissues and recorded any visible signs of decreased fitness, such as injuries, potential diseases (melanisations) or presence of parasites in examined tissues. We compared bacterial presence, load and diversity in hemolymph and hepatopancreas of 48 individuals by using 16S ribosomal DNA PCR.

Results: Our results show that 18.75% of individuals had bacteria present in the hepatopancreas, and 10.42% individuals in the hemolymph. Out of them, only 2 (4.2 %) individuals had bacteria present in both tissues. Individuals with bacteria recorded in the examined tissues had higher occurence of morphological signs of decreased fitness (75%) than individuals with no bacterial load in the tissues (55%).

PM197 IL-10-producing neutrophils: the unrecognized immune modulators in the Streptococcus pneumoniae lung infection

Liliana González[1], Loreani Noguera[1], Valentina Sebastián[1], Isidora Suazo[1], Bárbara Schultz[1], Omar Vallejos[1], Susan Bueno[1]

[1]Millennium Institute on Immunology and Immunotherapy, Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile., Región Metropolitana, Santiago, Chile

Background: Pneumococcal pneumonia is characterized by a high recruitment of neutrophils and excessive lung damage. Several reports have shown that besides their role in acute inflammation, neutrophils may also display anti-inflammatory properties. Data obtained in our laboratory show that neutrophils produce IL-10 during pneumococcal pneumonia. However, the relevance of these IL-10-producing neutrophils in response to *Streptococcus pneumoniae* infection remains unknown.

Objectives: 1. To determine the role of IL-10–producing neutrophils during pneumococcal pneumonia. 2.To evaluate whether *S. pneumoniae* directly induces the IL-10 production in neutrophils.

Methods: C57BL/6 wild type (WT) and IL-10-/- mice (the latter highly susceptible to *S. pneumoniae* infection), were intranasally transferred with WT or IL-10-/- neutrophils. 24 h later, mice were infected with $3x10^7$ *S. pneumoniae* CFUs. Lung infiltration and bacterial loads were evaluated after 1 day post-infection and a 10 days survival curve was performed. Further, neutrophils isolated from mouse bone marrow cells were exposed to *S. pneumoniae* molecular patterns and after 24 h post treatment the amount of IL-10 secreted to culture supernatants was evaluated by ELISA.

Results: The adoptive transfer of neutrophils improved IL-10-/- mice survival rate. In contrast, WT mice transferred with WT cells showed an increased clinical score and decreased survival. Furthermore, *S. pneumoniae* molecular patterns showed to be enough to induce high levels of IL-10 production in neutrophils. **Conclusions**. Our data show that neutrophils play a critical role during *S. pneumoniae* lung infection and suggest that IL-10 production by these cells, induced by bacteria, is crucial in disease outcome.

PM198 Human plague investigations identifies link to the hibernant marmot

Xin Wang[1], Huaiqi Jing[1]

[1]National Institute for Communicable Disease Control and Prevention of China CDC, Laboratory of Emergency, Beijing, China

Background: Yersinia pestis is an endemic zoonosis in Western China, particularly along with Qinghai-Tibetan plateau where Marmota. himalayana is the primary host. A herdsman died from bubonic plague and F1 antibody of one of the 10 contacts was positive without symptoms in Subei, Gansu Province, China, on December of 2017, when the marmot was in hibernation.

Objectives: To examine possible exposures of the human plague case and route of transmission to the contact.

Methods: A field investigation was conducted to identify possible exposures to plague infected animals. Serologic testing of F1 antibody was used to assess acute infection.

Results: Ten contacts of the male 45-year old deceased patient were quarantined and preventive medicated. One contact tested positive for F1 antibodies on Dec 14, 2017, started treatment with trimethoprim-sulfamethoxazole and tetracycline on Dec 12 and remained asymptomatic. Three shepard dogs belonging to the Y. pestis positive contact were also tested, with F1 antibody levels increasing 16-fold from December 17 to January 21, 2018, confirming infection in all three dogs. Investigation indicated that the confirmed case and the Y. pestis positive contact poached the hibernant marmot with Y.pestis together near their place of residence before their infection. The three dogs consumed raw marmot meat fed by their owner. The patient, contact and three dogs exposed simultaneously. It was a human plague event linked to the poaching and consumption of hibernant marmots.

PM199 Studies on antibiotic sensitivity pattern and biofilm formation potential of small colony variants of klebsiella pneumoniae

Dania Hassan[1;1], Omar Khalil[1], Michael Magaogao[1], Ashfaque Hossain[1]

[1]Rak Medical and Health Sciences University, Medical microbiology and immunology, Ras Al Khaima, United Arab Emirates

Background: Small colony variants (SCVs) are a slow-growing subpopulation of bacteria possessing distinct biological properties in comparison to the wild type (WT) parental bacteria. Biofilm is a collection of microorganism adhered to a living or nonliving surface, enclosed in a self-produced matrix of extracellular polymeric material. SCV formation and biofilm formation facilitates persistence of bacteria under stressful conditions and also in infected hosts. In this study, we investigated Gram negative pathogen Klebsiella pneumoniae for SCV formation and biofilm forming properties.

Objectives: To determine how the SCV of K. pneumonieae differ from their wild-type (WT) counterparts in terms of biochemical and metabolic characteristics and antibiotic resistance patterns. In addition, sensitivity to bactericiadal action of normal human serum and biofilm production potential of WT and SCVs were also compared.

Methods: Mueller-Hinton agar and broth were used to culture bacteria. Antibiotic sensitivity was assayed by disc diffusion and MIC determinations. For growth characteristics and serum bactericidal assays, standard procedures were used. Crystal violet dye binding spectrophotometric method and Congo red agar assay was used to investigate biofilm formation.

Results and Conclusion: SCV derived from K. pneumoniae strains were smaller in colony size, grew slowly, possessed identical biochemical properties (API), and serum sensitivity, exhibited increased resistance to gentamicin and increased biofilm formation potential in comparison to WT counterparts. These findings showing increased sensitivity to gentamicin and enhanced biofilm formation by SCVs in comparison to WT strains may have clinical implications. Whole genome sequencing of SCV and WT strains are in progress.

PM200 Antimicrobial Resistance Determinant in Genomes of Acinetobacter Clinical Isolates

Itziar Chapartegui-González[1], María Lázaro-Díez[1], Santiago Redondo-Salvo[2], Jose Ramos-Vivas[1]

[1]*IDIVAL, Microbiology, Santander, Spain*
[2]*IBBTEC, Santander, Spain*

Background: The importance of the genus Acinetobacter as the cause of nosocomial infectious has increased during the last years. A. baumannii has been associated with high morbidity and mortality rates. A. pittii is another opportunistic pathogen frequently isolated from Acinetobacter infections that has been less studied. The significant role of this bacterium in human infections, the emergence of resistant strains and its persistence in both hospital environment and devices have also become a great medical concert. Nowadays, the WGS analysis had improved our ability to understand the pathogen microevolution.

Objectives: To investigate and to compare the mechanisms of A. baumannii and A. pittii microbial resistance at the genetic level.

Methods: Genomic samples were extracted and purified using the GeneJet genomic DNA isolation kit (Thermo Scientific) from 6 A. baumannii and 4 A. pittii strains. The genomic DNA was submitted for Illumina sequencing. For genomes assembly and plasmid identification Unicycler and PLACNET software were used. Annotation and gene identification were done by using RAST, abricate, CARD and RGI databases.

Results: Between 3,566 and 3,881 proteins-coding-sequence were predicted in the whole genomes. All the strains carried at least one blaOXA gene and the chromosomic ampC gene. Six different allelic OXA-51-like forms were found in A. baumannii isolates; and OXA-58-like and OXA-213-like were found in all A. pittii isolates. Nine different aminoglycoside modifying enzymes genes were also found. All the strains harbored efflux pump adeIJK, while all the A. baumannii strains had the adeFGH, but only half of them had the adeABC operon.

PM201 Unveiling the outer membrane vesicle proteome of the fish pathogen A. salmonicida

Tobias Kroniger[1], Anke Trautwein-Schult[1], Bernd Köllner[2], Dörte Becher[1]

[1]*University of Greifswald, Department of Microbial Proteomics, Greifswald, Germany*
[2]*Friedrich-Loeffler-Institut, Institute of Immunology, Greifswald - Insel Riems, Germany*

Background: Aquaculture is one the most rapidly growing food sectors globally. Still, due to bacterial infections, high economic losses occur. In this study we utilized mass spectrometry based proteomics to study the physiology of one of these pathogens: *Aeromonas salmonicida*.

Objectives: Outer membrane vesicles (OMVs) are produced by all bacteria and are involved in a variety of cellular functions. Anyhow, the knowledge of the cargo of *A. salmonicida* OMVs is very limited. To detect changes in the OMV proteome, we compared the OMV composition when the bacteria were grown in two different growth media.

Methods: *A. salmonicida* was grown in lysogeny broth liquid medium and a chemically defined medium without iron and calcium supplements to mimic host conditions. For a global overview, cytoplasmic, inner membrane, outer membrane and extracellular proteins as well as proteins of outer membrane vesicles were enriched by subcellular fractionation and subsequently analyzed in a mass spectrometer.

Results: Overall, approximately 40% of the proteins predicted were identified. The comparison of the predicted cellular locations of the proteins and the subcellular fractions in which the proteins were identified showed a clear enrichment of the proteins according to their prediction. Interestingly, the most striking changes were detected in the protein composition within the OMV fraction, indicating that different sorting or biogenesis mechanisms were utilized.

PM202 Investigation on Small Colony Variants of Escherichia coli

Kendah Azzam[1], Balkes Azzam[1], Sheimaa Hassaid[1], Michael Magaogao[1], Ashfaque Hossain[1]

[1]RAK Medical and Health Sciences University, Medical Microbiology, Ras Al Khaimah, United Arab Emirates

Background: Small Colony Variants (SCVs) are as defined, smaller variants of bacterial colonies possessing distinct morphological, physiological and biochemical properties from the wild type of the same bacterial strain. SCVs are reported to cause chronic infections by different pathogens. In this study, we investigated the SCV of ATCC 25922 *E.coli* strain and a clinical isolate of *E. coli.*

Objectives: The objectives of the present study were to compare the growth characteristics, biochemical properties and antibiotic sensitivity pattern of the parent and the SCV variants of the *E. coli* strains.

Methods: SCVs were grown on Mueller Hinton Agar (MHA) or modified Muller-Hinton agar containing different concentrations of a stress factor; water extract of black seed, Nigella sativa). Sensitivity to different antibiotics was determined using Kirby-Bauer disc diffusion method. Growth characteristics and sensitivity to bactericidal action of serum was determined using standard procedures. API was used for biochemical property tests.

Results: SCV showed smaller colony size and less colony number while growing on MHA or modified MHA. SCVs also exhibited slower growth rate but identical biochemical properties and sensitivity to serum killing. Zones of inhibition using Kirby-Bauer method showed almost same results for aztreonam, ceftazidime, ciprofloxacin, ampicillin and meropenem as WT parental strain. However Gentamicin zone of inhibition to SCV was markedly lesser in diameter in comparsion to that of WT indicating that SCV developed resistance to Gentamicin, which is of great clinical significance. Studies are ongoing aiming at further characterization of the SCVs.

PM203 Study of Group A Streptococcus virulence using a highly necrotising clinical isolate

Geoffrey Deneubourg[1], Catherine Neerdaels[1], Dalila Lakhloufi[1], Valérie Delforge[1], Mark Davies[2], Pierre Smeesters[1], Anne Botteaux[1]

[1]*Université Libre de Bruxelles, Molecular Bacteriology Lab, Anderlecht, Belgium*
[2]*Doherty Institute, Melbourne, Australia*

Background: Group A *Streptococcus (*GAS) causes more than 500.000 deaths per year. GAS is responsible for a broad spectrum of diseases ranging from pharyngitis to life-threatening invasive diseases. GAS virulence is due to a complex interplay between host factors and bacterial factors, including DNases and proteases.

Objectives: To investigate the contribution of DNases to virulence and pathogenesis of invasive GAS disease using an isolate recovered from a severe clinical presentation. As the clinical isolate (called L01) has 4 DNases (Sdn, Spd1, Spd3 and Spd4), we want to investigate their individual role in the L01 pathology, as well as their potential cumulative effect. We also want to see if other factors are involved in L01 pathogenesis.

Methods: We have cloned and produced 3 of these DNases (Sdn, Spd1, Spd3) and their mutated version as recombinant proteins. We have tested the L01 fitness and the competition with other bacteria by CFU enumeration. We have also monitored the expression of these DNases by RT-qPCR in different conditions. Finally, we have tested the strain ability to degrade elastin and collagen in enzymatic assays.

Results: We have shown that the 4 DNases, even if all expressed in the exponential phase, are more expressed during the stationary phase. Three of them are active DNases and we have mutated their catalytic residue. We have also shown that the presence of exogenous DNA gives a fitness advantage to the L01 in comparison with the L01 without DNA.

PM204 Interactions of Helicobacter pylori cysteine-rich protein HcpE with gastric cells

Carole Creuzenet[1], Noor Salloum[1], Andrew MacDonald[1], Elyse Roach[2], Cezar Khursigara[2]

[1]*Western University, London, Canada*
[2]*University of Guelph, Guelph, Canada*

Background: *H. pylori* causes gastric ulcers and cancers but the mechanisms are not fully understood. It secretes proteins which may elicit gastric inflammation, including the Helicobacter cysteine rich protein HcpE. HcpE is exclusively comprised of Sel-like Repeat (SLR) motifs known to be involved in protein/protein interactions. We hypothesize that secreted HcpE interacts with a host protein via its SLR motifs to exert its effects.

Objectives: To determine the function of HcpE and the underlying mechansims, we will determine its effects on gastric cells and identify host cell proteins that HcpE interacts with to mediate theses effects.

Methods: We use human AGS gastric cells to monitor the effect of HcpE on gastric cell viability (MTT assay), inflammatory signaling (ELISA) and morphological changes (microscopy) after exposure to wild-type or *hcpE* knockout mutant *H. pylori,* their culture supernatants or purified HcpE. We use Immuno Electron Microscopy (IEM) to detect intracellular HcpE in gastric cells. Finally, we use BirA-mediated *in situ* biotinylation and Western blotting to detect intracellular host partners.

Results: Spent culture supernatants of *H. pylori* induced production of interleukin 8 in AGS cells in a HcpE- and dose- dependent manner. While no effects on gastric cell viability was noted, cellular elongation was reduced in the *hcpE* mutant. Intracellular HcpE was detected by IEM and an HcpE-specific intracellular partner was detected in AGS cells after transfection with HcpE-BirA. Identifying this partner will allow assessing the functional outcome of the interaction by RNA interference.

PM205 Adaptation of Brucella spp. to acid stress at pH 4.5: A transcriptomic approach

Jorge de la Garza[1], Alessandra Occhialini[1], Stephan Köhler[1]

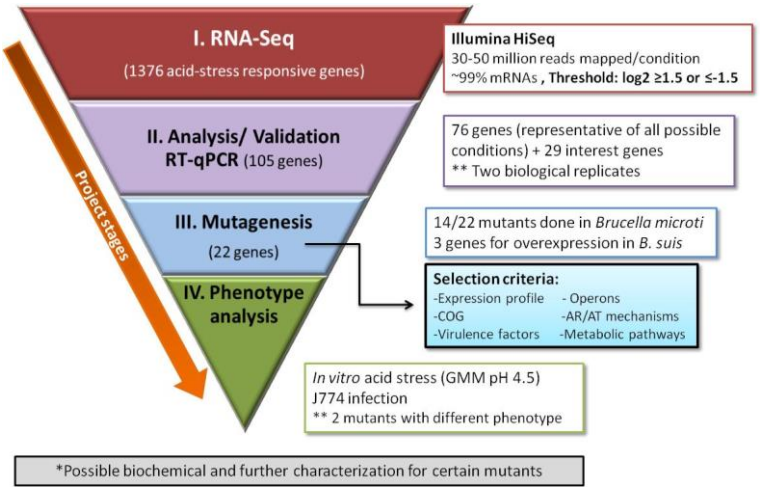
[1]*Institut de Recherche en Infectiologie de Montpellier (IRIM), Montpellier, France*

Background: *Brucella* spp "atypical" species are phenotypically and biochemically different from "classical" species, despite their common genetic background, and their isolation from wildlife reopened the debate on brucellosis reemergence. *Brucella microti,* closely related to *Brucella suis*, is a fast growing, acid-resistant bacterium lethal in mice and a useful model for classical/atypical species comparisons. Its acid resistance phenotype might have important implications allowing bacterial survival in gastrointestinal tract or acidified intramacrophagic vacuoles, required for pathogenesis.

Objectives: To identify the factors shaping *B. microti* acid resistance at pH 4.5 in comparison to the classical species *B. suis*, its closest relative, using a transcriptomic approach.

Methods: High throughput RNA-Seq analyses of *B.suis* and *B.microti* exposed to pH 4.5 and 7.0 were performed. Genes significantly up/downregulated were identified using a log2-fold change >1.5/<-1.5 threshold. For validation, the expression of 105 genes was assessed by RT-qPCR, using 2 reference genes (80% average validation score). Functional characterization of significant genes was performed (virulence factors, transporters, transcriptional regulators, etc.) and 22 genes were chosen for mutagenesis in *B. microti* and/or *B. suis*, for phenotypical testing.

Results: 1376 genes were identified as acid-stress responsive genes. From these, 235 and 285 genes were specific for *B. microti* and *B. suis*, respectively, while 855 genes were responsive in both strains. These acid-stress regulated genes encoded 69 virulence factors, 7 Type IV Secretion System effectors, 38 LPS/cell wall-related factors, 160 ABC-transporters and 67 transcriptional regulators, as well as 15 pseudogenes (all in *B. suis*). Several mutants showed acid-sensitive phenotypes *in vitro*.



PM206 Attenuating virulence of Vibrio species by targeting the conserved transcriptional regulator HlyU

Sang Ho Choi[1], Zee-Won Lee[1], Byoung Sik Kim[2]

[1]Seoul National University, Department of Agricultural Biotechnology, Seoul, Korea, Rep. of South
[2]Ewha Woman's University, Department of Food Science & Engineering, Seoul, Korea, Rep. of South

Background: Increasing antibiotic resistance has led to the development of new strategies to combat bacterial infection. Anti-virulence strategies that impair virulence of bacterial pathogens are one of the novel approaches with less selective pressure for developing resistance than traditional strategies that impede viability.

Objectives: To inhibit the virulence of *Vibrio* species including a fulminating human pathogen *Vibrio vulnificus*, we have identified a small molecule CM14 that inhibits HlyU, a key transcriptional regulator required for the activation of various virulence genes.

Methods: We performed high-throughput screening of 8,385 compounds and identified CM14 that inhibits the activity of HlyU. Effects of CM14 on virulence of *V. vulnificus* were examined *in vitro*, *ex vivo*, and *in vivo*. Structural, biochemical, and mass spectrometric analyses demonstrated a mechanism of CM14 that interferes with the DNA-binding activity of HlyU by inducing a conformational change via covalent modification of the Cys30 residue.

Results: CM14 reduces HlyU-dependent expression of virulence genes in *V. Vulnificus*, thereby decreasing hemolytic activity against human erythrocytes and impeding cytopathicity and cytotoxicity toward human epithelial cells. Notably, CM14 significantly enhances survival of mice infected with *V. vulnificus* by alleviating hepatic and renal dysfunction and systemic inflammation. Furthermore, CM14 decreases the expression of various virulence genes of other *Vibrio* species and thus attenuates their virulence phenotypes. Since CM14 is not toxic toward bacteria, human cells, and mice, this molecule could be an anti-virulence agent against HlyU-harboring *Vibrio* species with a low selective pressure for the emergence of resistance.

PM207 Binding interference between Bartonella Adhesin A and Fibronectin as a novel therapeutic concept

Diana Vaca[1], Arno Thibau[1], Johan Malmstrom[2], Lotta Happonen[2], Volkhard A. J. Kempf[1]

[1]*Goethe University Hospital, Institute of Medical Microbiology, Frankfurt, Germany*
[2]*Lund University, Division of Infection Medicine, Lund, Sweden*

Background: *Bartonella henselae* is a facultative intracellular bacterium, responsible for various human diseases like cat scratch disease and vascular proliferations (bacillary angiomatosis). *Bartonella* adhesin A (BadA), a trimeric autotransporter adhesin, is a major pathogenicity factor of *B. henselae* mediating bacterial adherence to endothelial cells and extracellular matrix (ECM) proteins. The identification of specific binding sites between BadA and ECM proteins might give insights about the use of BadA-specific antibodies as anti-ligands to treat bacterial infections by a "new class" of antibiotics

Objectives: The project aims the detailed analysis of fibronectin and BadA binding as the basis of the interaction between BadA and ECM proteins in host-cell adhesion processes, and the design of adhesion-inhibiting peptides for later anti-ligand application

Methods: *B. henselae* strains (wildtype and BadA deficient) are exposed to fibronectin and human endothelial cells to study binding interactions using *in vitro* infection models. A broad screening of fibronectin binding sites is performed using enzymatic digestion and standardized *in vitro* binding assays. The interaction between fibronectin and BadA is analyzed using mass spectrometry. As further steps, fibronectin will be genetically modified (e.g. deletion/modification of the identified binding domains) as a test of function. Finally, after the understanding of the adhesion process, synthetic molecules will be generated for the inhibition of bacterial adhesion.

Results: We expect the definition of certain fibronectin domains involved in the bacterial adhesion process and the generation of artificial peptides for bacterial adherence inhibition to host cells (anti-ligands).

PM208 Analysis of Vibrio cholerae possessing ctxA gene from environmental water in Kolkata

Eizo Takahashi[1], Goutam Chowdhury[2], Asish Mukhopadhyay[2], Shin-ichi Miyoshi[3], Keinosuke Okamoto[1]

[1]Collaborative Research Center of Okayama University for Infectious Diseases in India, Okayama University, Kolkata, India
[2]National Institute of Cholera and Enteric Diseases, Kolkata, India
[3]Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University, Okayama, Japan

Background: The virulence of *Vibrio cholerae* is basically determined by the production of cholera toxin (CT). Although there are 206 serotypes in *V. cholerae,* CT-positive and pathogenic strains are restricted to only two serotypes, O1 and O139. As Bengal region in which Kolkata is located has been said to be the place of origin of virulent *V. cholerae*, it is likely that new virulent strains generate from environment water in this area. It is necessary to check the birth of new virulent strain to protect people from damage by virulent *V. cholerae*.

Objectives: Previously we have performed the comprehensive survey on *V. cholerae* inhabiting environmental water in Kolkata. Six strains of *V. cholerae* possessing cholera toxin gene (*ctx*) have been identified, and four of them are NAG strains. The characteristics of environmental NAG strains possessing *ctx* gene has remained unclear. In this study, we examined the pathogenic property of these NAG vibrios.

Methods: To investigate the production of CT of environmental strains, the amount of CT in culture supernatant was quantified by GM1-ELISA. The transcriptional level of *ctxA* was evaluated using quantitative RT-PCR. To investigate the pathogenicity of *V. cholerae,* enterotoxicity was assessed using rabbit ileal loop assay.

Results: The production of CT of environmental NAG vibrios was very low and/or negligible. Coincidentally, the transcriptional level of *ctxA* of these strains was extremely low. However, a NAG strain apparently exhibited the enterotoxicity. The enterotoxicity by NAG vibrio was suppressed by anti-CT antiserum, indicating that this strain may be pathogenic.

PM209 Impact of Long-Term Prophylaxis on Bladder Colonisation by Escherichia coli in CISC Patients

Aaron Ming Zhi Tan[1], Maxime Vallée[1;2], Catherine Mowbray[1], Holly Fisher[3], Christopher Harding[4], Judith Hall[1], Phillip Aldridge[1]

[1]Newcastle University, Institute for Cell and Molecular Biosciences, NEWCASTLE-UPON-TYNE, United Kingdom
[2]CHRU Nantes , Service d'Urologie et de Transplantations rénales, Nantes, France
[3]Newcastle University, Institute for Health and Society, NEWCASTLE-UPON-TYNE, United Kingdom
[4]Newcastle upon Tyne NHS Foundation Trust, Urology, NEWCASTLE-UPON-TYNE, United Kingdom

Background: The uropathogenic *Escherichia coli* (UPEC) is responsible for causing between 60 to 80% of all UTI cases. The AnTIC study was a randomised, open-label, superiority trial which compared the antibiotic prophylaxis versus short term antibiotic treatment for recurrent UTI prevention in clean intermittent self-catheterisation (CISC) patients.

Objectives: Our aim was to genetically define AnTIC UPEC isolates and correlate bacterial motility, a UTI virulence factor, and antibiotic use asking: 1) does antibiotic treatment select specific genetic subgroupings? 2) what is the nature of multi-drug resistance (MDR) development? and 3) Does the genetics of motile strains aid our understanding of the observed clade structure and antibiotic use?

Methods: The AnTIC bio-bank of bacterial isolates generated during the RCT phase was the source of 96 random UPEC isolates that were genotyped. A phylogenetic tree was generated from the sequence data obtained. Motility was assessed using motility agar assays and correlated to clade data. We exploited next-generation sequencing technology to sequence specific samples from our cohort, based on motility and antibiotic resistance profiles.

Results: AnTIC *E. coli* showed a bias toward clades B2 and D. Analysis for MDR amongst UPEC isolates has led to ongoing whole genome sequencing of AnTIC isolates and clade D strains. Our work provides a valuable and unique insight of UPEC isolated during a CISC based clinical trial. The greater frequency of clade D may reflect the source of the isolates. Our whole genome sequence analysis will improve our understanding of host-microbe interactions during CISC use.

PM210 Investigations into a novel hypothetical virulence factor from Group A streptococcus

Haniyeh Aghababa[1], Jacelyn Jacelyn Loh[1], Thomas Proft[1], Paul Young[2]

[1]*The University of Auckland, Molecular Medicine and Pathology, New Zealand*
[2]*Auckland university, Biological Sciences*

Background: Group A Streptococcus (GAS) is a major human pathogen that causes a range of diseases, from minor skin and throat infections such as impetigo and pharyngitis to severe invasive infections such as streptococcal toxic shock syndrome. This is facilitated by a large arsenal of virulence factors that contribute to colonisation of host tissue, invasive spreading, and immune evasion.

Objectives: To functionally characterise the putative streptococcal virulence factor Spy0136 and analyse its suitability as a target for therapeutic intervention or vaccine development.

Methods: The complete spy0136 gene was amplified from GAS SF370 by PCR and cloned into a pProEx-Hta expression vector. The recombinant protein was expressed as a maltose binding protein (MBP) fusion in *E. coli* BL21 and purified by immobilised metal chelate affinity chromatography (IMAC) using NTA-Ni2+ resin. The MBP was removed by cleavage with recombinant tobacco etch virus (rTEV) protease and further purified by size exclusion chromatography.

Purified rSpy0136 was investigated for specific binding to host factors using pull-down experiments with human plasma. In addition, the effect of Spy0136 on complement was evaluated using the 50% Complement Haemolytic Assay (CH50) and the Wielisa total complement system screen (Wieslab).

Results: Spy0136 can be produced as a soluble recombinant protein which binds to human plasma proteins. It demonstrated inhibition of complement-mediated lysis of sheep red blood cells in a CH50 assay and reduced the activity of all three complement pathways in a dose-dependent manner.

Preliminary results suggest Spy0136 is a novel immune evasion factor that interferes with the complement system.

PM211 How do regulatory non-coding RNA dictate the outcome of Enteropathogenic E. coli infection?

Zakhar Krekhno[1], Brett Finlay[1]

[1]*The University of British Columbia, Michael Smith Laboratories, Vancouver, Canada*

Background: Diarrheal disease caused by Enteropathogenic *E. coli* (EPEC) is a leading cause of mortality in children under the age of two, particularly in developing countries. Moreover, the frequency of antibiotic resistance in EPEC infections is rising. Both human and bacterial transcriptomes change drastically in response to altered environmental conditions, therefore, monitoring such changes during infection could uncover novel drug targets. In particular, both human and bacterial non-coding RNA (ncRNA) have been previously shown to modulate infections.

Objectives: Thus, I hypothesize that ncRNAs are vital determinants of the EPEC infection outcome. I first aim to identify ncRNAs induced during an EPEC infection, and then assess their capability to act as novel antimicrobial targets.

Methods: RNA-sequencing was used to assess both bacterial and human transcriptomes in an *in vitro* model of infection at several timepoints representing “early”, “stable”, and immediately preceding cell death. Gene co-expression analysis in addition to *in silico* ncRNA target prediction were used to characterize novel ncRNA. Reverse transcription quantitative polymerase chain reaction (RT-qPCR) was used to validate RNA-sequencing results.

Results: The RNA-sequencing revealed a low transcriptional response from the host most likely due to the swift nature of infections (<3 hours). Conversely, EPEC transcriptome underwent drastic changes both in coding and non-coding RNA expression. ~20 small ncRNA genes were significantly differentially expressed over the course of infection. These were predicted to regulate a wide variety of genes ranging from general metabolism to pathogenic proteins. Overall, these genes could potentially act as novel anti-EPEC drug targets.

PM212 Quantitative Affinity-Purification And Surface-Adsorption Mass Spectrometry To Map Streptococcus pyogenes And Human Protein Interaction

Sounak Chowdhury[1], Lotta Happonen[1], Dirk Linke[2], Johan Malmstrom[1]

[1]*Lund University, Faculty of Medicine, Department of Clinical Sciences, Division of Infection Medicine, Lund, Sweden*
[2]*University of Oslo, Molecular Microbiology, Oslo, Norway*

Background: *Streptococcus pyogenes* (*S. pyogenes*), a human specific gram-positive pathogen, is associated with considerable morbidity and mortality. M proteins are the major virulence factor on the surface of *S pyogenes* facilitating it to evade the adaptive and innate immune systems. Till date, 223 different M proteins have been reported which are to a varying degree associated with invasive diseases. The differential propensity to cause invasive disease's may be coupled to the multifunctional nature of the M-proteins and the proteins ability to interact with numerous human proteins, that still remains unidentified.

Objectives: The objective of this study was to unbiasedly identify and map protein-protein interaction between different M proteins and strains of *S. pyogenes* with human proteins formed in different kinds of invasive diseases.

Methods: To address the objectives, we combined 'Protein-Centered' Affinity-Purification–Mass Spectrometry(AP-MS) and 'Bacterial-Surface Centered' Surface-Adsorption (SA)-MS approach, to quantitatively define host-pathogen interactions occurring in *S.pyogenes* serotypes responsible for invasive diseases. To compare the levels of specific human proteins binding to different M proteins or the bacterial surface, affinity-tagged M proteins and different bacterial strains were enriched to determine interacting host proteins followed by quantitative mass spectrometric analysis.

Results: The combined affinity-capture and surface adsorption MS approaches, help us identify and quantify significant human proteins interacting with different strains of *S.pyogenes*. We anticipate that the results will determine proteins that play a pivotal role in the development of the invasive infections, and be the starting point for the design of new therapeutic approaches against *S. pyogenes* infection.

PM213 Seroepidemiology and genetic characterization of Orientia tsutsugamushi from Nepali Typhus Patients

Sagar Aryal[1], Anurag Adhikari[1], Ram Deo Pandit[2], Birendra Prasad Gupta[2]

[1]*Kathmandu Research Institute for Biological Sciences, Lalitpur, Nepal*
[2]*Institute of Medicine, Aayurved Campus, Kirtipur, Nepal*

Background: Scrub typhus is caused by the *Orientia tsutsugamushi* which is an obligate intracellular bacterium, transmitted to human by bite of infected mite vector.

Objectives: The aim of this study is to identify genotypes of *O. tsutsugamushi* strains circulating in Nepal.

Methods: The prospective cross-sectional study was conducted among the patients visiting the Sagarmatha Diagnostic Pathology & Research Center at Nepalgunj, Bheri district of west Nepal, during a 6-month period (July 2016 to December 2016). The patients' (n=122) acute phase sera and buffy coat (collected within 1-6 days of illness onset); frozen at -20°C were used for the pathogen DNA isolation and ELISA for Scrub Typhus IgM. Nested-polymerase chain reaction (N-PCR) and real-time PCR (Q-PCR) were performed for the diagnosis of scrub typhus.

Results: Out of 122 scrub typhus suspected patients, 116 (95.08%) had IgM ELISA against *O. tsutsugamushi*. The 47 kDa N-PCR produced 500 bp amplicons in 18 plasma specimens. The resulting fragment were sequenced by sanger sequencing using *O. tstsugamushi* genotype specific primers. Phylogenetic trees were constructed by comparing 47 kDa sequences (500 bp) of the fourteen (n=14) positive specimens and reference strains of the *Orientia* genus and *Rickettsia* genus from GenBank. All the detected isolates in this study fall within the same clade of *O. tsutsugamushi*. The N7, N30, N27, N12, N36, N18, N17, N9, N5, N11, N13, N21 isolates were phylogenetically close to *O. tsutsugamushi* Karp strain, and the 796 & 836 isolates were close to *O. tsutsugamushi* Kuroki strain.

PM214 Cellular study of a bacterial DNA-damaging virulence factor, the Cytolethal Distending Toxin

Benoit Pons[1;2;3], Julien Vignard[1], Elizabeth Bezine[1], Saleha Hashim[1;3], Valérie Lobjois[2], Bernard Ducommun[2], Gladys Mirey[1;3]

[1]Toxalim. INRA laboratory, Toulouse, France
[2]ITAV USR3505 CNRS, Toulouse, France
[3]Université Toulouse Paul Sabatier, Toulouse, France

Background: Genotoxic agents alter the genetic information by damaging DNA inside living cells, leading to genetic instability and carcinogenesis. Several bacteria from the human microbiota produce genotoxic virulence factors, called genotoxin. Among these genotoxins, the Cytolethal Distending Toxin (CDT) is produced by several pathogenic bacteria. In the digestive tract, CDT modulates the immune response, induces pro-inflammatory signals and disturbs the epithelial barrier which may result in chronic infection and inflammation. Moreover, CDT has been indirectly associated with higher colorectal cancer risk. Indeed, some CDT-producing bacteria have been found around human colorectal tumours. In addition, several CDT were shown to induce several cancerous markers in mice. On cellular level, CDT induces DNA breaks, disturbs the cell cycle and may lead to cell senescence or cell death.

Objectives: CDT has two distinct biochemical activities: DNase and phosphatase. Overall, the influence of these two activities on CDT toxicity and physiopathology is not characterised to this day. Our objective is therefore to study the toxicity of CDT in link with its biochemical activities.

Methods: We have therefore developed i) CDT mutations aiming to disturb one of the two activities without hampering the other and ii) cellular assays that allow evaluating and comparing the activities of different CDTs. We interestingly obtained CDT mutants impacting specific CDT's activities.

Results: Overall, through cellular approaches, we better characterised CDT mechanism of action as a first step to get more insight on the effects of the toxin on human health, particularly on genetic stability, intimately linked to carcinogenic development.

PM215 How are A. fumigatus conidia protected against peroxides?

Esther Keizer[1], Michelle Schinkel[1], Han Wösten[1], Hans de Cock[1]

[1]*Utrecht University, Biology, Utrecht, Netherlands*

Background: Fungi protect themselves against ROS components using superoxide dismutates, catalases and melanin. DHN-melanin in the cell wall of conidia of *A. fumigatus* and catalases were previously shown to be involved in conidial-peroxide protection. Here we investigated their role in more detail.

Objectives: Elucidate the mechanisms of conidia of *A. fumigatus* to protect themselves against peroxide.

Methods: Peroxide-plate assays showed increased peroxide sensitivity of a *pksP* (UV mutant) as compared to its WT background (ATCC46645) and, remarkably, the *pksP* complemented strain was not rescued. Interestingly, in this assay no difference in peroxide sensitivity was observed between a *pksP* deletion mutant and its WT (CEA10). However, when dormant conidia of these WT and *pksP* mutants were directly exposed to peroxide in solution no difference in sensitivity was observed. Analysis of gene expression of different catalase genes in dormant conidia using qPCR indicate marked differences between these strains.

Results: Expression of the *catA*, *cat*1 and *cat*2 genes was strongly increased in CEA10 Δ*pksP* in contrast to ATCC Δ*pksP* mutant. These results suggest that absence or presence of DHN-melanin and expression of catalases in dormant conidia is not correlated with conidial-peroxide sensitivity since no difference was observed in direct peroxide exposure in solution. However, differences in catalase gene expression might explain the differences in peroxide sensitivity between the two different *pksP* mutants in the plate assay.

PM216 Ironing clothes: ancient weapon against typhus?

Hector M. Díaz-Alejo[1], Paloma Martinez-Alesón[1], Eduardo Costas[1], Victoria López-Rodas[1], Beatriz Baselga Cervera[1], Camino García-Balboa[1]

[1]*School of Veterinary Medicine, Animal Sciences, Madrid, Spain*

Background: Whilst being an energy waste and annoying for the majority of the population, ironing is a widespread task. People usually iron in order to straighten clothes. We propose that the social predilection for smooth could be regarded from another perspective. Louse-borne infectious diseases, despite the advent of hygiene, remains a main public health concern. Lice and other parasites are vectors of diseases such as typhus. In this work, we investigated the possible relationship between ironing and louse-borne diseases.

Objectives: We tried to unveil the point of ironing on a historical and sanitary context, focused on its use against body lice and prophylactic against louse-borne diseases.

Methods: We carried an online meta-analysis, searching for people´s stated reasons for ironing nowadays. A bibliographic revision to seek for hints on the historical expansion and reasons of its use was also carried. In order to determine whether the hypothesis is accurate or not, an indirect approach is currently undergoing.

Results: Apparently, modern irons appeared in the late Middle Age. In this period also the first typhus outbreaks were documented. The extreme heat would kill instantly any vegetative bacteria on clothes and the shape would let it reach the seams, where the nits are. The First World War was the major event where these diseases appeared. In the years following this war, ironing reached its peak with large-scale sales. Further investigation of this study may confirm our hypothesis.

PM217 Detection of a new sequence type of Leptospira interrogans in a patient with myasthenia gravis

Mateusz Markowicz[1], Matthias Tomschik[2], Inga Koneczny[2], Anna-Margarita Schoetta[1], Friedrich Zimprich[2], Gerold Stanek[1]

[1]Institute for Hygiene and Applied Immunology, Centre for Pathophysiology, Infectiology and Immunology, Medical University of Vienna, Vienna, Austria
[2]Department of Neurology, Vienna, Austria

Background: Leptospirosis is a zoonotic disease, caused by spirochetes of the genus *Leptospira*. The bacteria are spread by the urine of infected animals. Leptospirosis causes nonspecific symptoms including fever, chills and headache; in some cases severe courses with kidney and liver failure may develop.

Myasthenia gravis is an acquired, antibody-mediated autoimmune disease causing muscle weakness and increased fatigability. We present a case of acute leptospirosis neurological symptoms consistent with myasthenia gravis. A new sequence type of *L. interrogans* was identified in the blood of the patient.

Objectives: A previously healthy 32 years old Austrian traveler returned from South-East Asia and he presented with generalized weakness, malaise, double vision, and a history of fever and diarrhea in the past two days. Three days before the onset of symptoms he had returned from a 4-week vacation in Vietnam and Thailand. Neurological examination confirmed MG. *L. interrogans* was detected in the blood of the patient followed a phylogenetic analysis of the isolate.

Methods: Realtime PCR on EDTA-blood targeting the gene for the major outer membrane protein lip L32 of human pathogenic Leptospira spp. was positive. Seven housekeeping genes of *Leptospira* spp. were sequenced and analyzed for multilocus sequence typing. Among these we found two new alleles which resulted in a new sequence type (ST). A phylogenetic tree was constructed for all 147 currently available *L. interrogans* STs, including our newly discovered ST247.

Results: We report a new sequence type of *L. interrogans* associated with myasthenia gravis which is an uncommon presentation of leptospirosis.

PM218 Phylogenetic status and host range of plant pathogen Pseudomonas syringae isolated from sugar beet fields in Serbia

Ivan Nikolić[1], Cindy Morris[2], Tanja Berić[1], Jelena Lozo[1], Đorđe FIra[1], Slaviša Stanković[1]

[1]*Faculty of Biology, University of Belgrade, Belgrade, Serbia*
[2]*INRA, Unite de Pathologie Vegetale, Monfavet, France*

Background: *Pseudomonas syringae* is a very heterogeneous bacterial species, inhabiting various ecological niches, affecting numerous host plants and representing great phylogenetic diversity. In 2013, a leaf spot disease of sugar beet caused by *P. syringae* was detected for a first time in Serbia.

Objectives: We aimed to determine the phylogenetic diversity of isolates from sugar beet and the potential host range in order to define status of isolates within *P. syringae* species complex and estimate the pathogenic diversity.

Methods: Twenty representatives among 104 collected *P. syringae* isolates from commercial fields of sugar beet were tested. Sequencing of *cts* gene, sequence analysis by the Neighbor-joining method and comparison with 59 *P. syringae* strains from 13 phylogenetic groups was performed for all tested isolates. The host range was determined by leaf inoculation of 16 different plant species in controlled greenhouse conditions. The virulence and aggressiveness of tested isolates were measured.

Results: Phylogenetic analysis showed that all tested *P. syringae* isolates from sugar beet belong to the 02b phylogroup within the *P. syringae* species complex, representing one of the most abundant *P. syringae* phylogroups in the environment. Host range tests revealed extremely aggressive isolates causing symptoms on every tested plant (70% of tested isolates) as well as weakly aggressive isolates (30% of tested isolates). These findings provide the first insight to status of collected isolates from Serbian sugar beet fields within *P. syringae* metapopulation and provide information about potential risk to other crops.

PM219 Antibiotic profile of klebsiella pneumonia isolated from sputum samples in hospitalized patients in southern nigeria

Rachael Osagie[1], aidevbo eyaufe[1], faith unuabonah[2]

[1]*ambrose alli university, medical microbiology, ekpoma, Nigeria*
[2]*ambrose alli university, medical laboratory science, ekpoma, Nigeria*

Background: This study aimed at isolation, identification and determination of antibiogram of *Klebsiella pneumoniae* isolates from other pathogens in sputum samples from hospitalized adults in 5(five) communities referral hospital in Edo- Nigeria. Clinical sample collection was done over a period of six (6) months from both male and female patients

Objectives: To screen for antibiotic of choice for *K.pneumoniae* infection among hospitalized patients.

Methods: Identification of test strain was by growth on Selective medium; m-FC agar, API20E and PCR for resistant strains and while XLD and MacConkey agar were used for purity test. Antibiotic susceptibility testing was done by Kirby-Bauer disc diffusion method and interpreted using CLSI guidelines, Statistical analysis was based on $p<0.05$.

Results: A total number of 70 pathogens were isolated from 500 samples collected. Out of the 70 pathogens, 31 (44.3%) were *Klebsiella pneumoniae.* Isolation rate was higher in males (54.8%) than females (45.2%) and in 20-29yrs old subjects (35.5%),there was no statistically significance of *K. pneumonia* occurrence among the different sex at $p>0.05$ ($p=0.306$) or age group $p=0.141$. There was a statistic significance $p<0.05$ ($p=0.0397$) between subjects without history of antibiotic administration for one (1) year prior to admission compared to subjects who had had one or more antibiotic course within that year. Susceptibility of *Klebsiella pneumoniae* was observed with levofloxacin (100%), (96.8%) to imipenem, (93.5%), ciprofloxacin and gentamicin (87.1%). intermediate resistance to Streptomycin (35.5%), high antibiotic resistance to ampicillin (80.6%), amoxicillin (74.2%), two isolates were resistant to imipenem (65%) with plasmid band at 800bp and 400bp...

PM220 Ascorbic acid modify the amoebic liver abscess development in two rodent animal models

Rosa Adriana Jarillo-Luna[1], Luz María Cárdenas-Jaramillo[2], Andrea Cruz-Baquero[3], Rafael Campos-Rodríguez[4], Judith Pacheco-Yepez[5]

[1]Escuela Superior de Medicina, Instituto Politécnico Nacional, Coordinación de Ciencias Morfológicas, CDMX, Mexico
[2]Escuela Superior de Medicina, Instituto Politécnico Nacional, Coordinación de Ciencias Morfológicas, Ciudad de México, Mexico
[3]Facultad de Ciencias de la Salud, Universidad Colegio Mayor de Cundinamarca, Departamento de Bacteriología, Bogotá, Colombia
[4]Escuela Superior de Medicina, Instituto Politécnico Nacional, Sección de Estudios de Posgrado e Investigación, Ciudad de México, Mexico
[5]Escuela Superior de Medicina, Instituto Politécnico Nacional, Sección de Estudios de Posgrado e Investigación, CDMX, Mexico

Background: *Entamoeba histolytica* (*Eh*) causes the amoebic liver abscess (ALA). Studies have been conducted in susceptible and resistant animal models (hamsters and mice). After arrival of amoebae to the liver, a lysis of inflammatory infiltrate and the generation of free radicals are observed. Neutrophils myeloperoxidase (MPO) produces the cytotoxic molecule, HOCl. Ascorbic acid (AsA) removes HOCl and it may decrease the extent of liver damage in ALAs of animal models.

Objectives: Since the HOCl may participate in the tissue damage, we analyzed the effect of ascorbic acid on the damage percentage, MPO and neutrophils presence in the ALAs of animals treated with AsA.

Methods: Hamsters and mice were administrated with AsA every 24 hours during 3 days before inoculating them with *Eh* (AsA group). The control group did not receive AsA before inoculation. Animals were sacrificed at 3, 6 and 12 h post-inoculation. ALA´s samples were taken, and the percentage and characteristics of hepatic lesions were determined. The number of neutrophils was quantified and MPO presence was analyzed.

Results: AsA treatment in both models induced decrease in the percentage of ALAs at different times of ALA evolution, as well as changes in the number of neutrophils and in the number of amoebae damaged in ALAs. The presence of MPO was not modify. Ascorbic acid removed the HOCl present in the ALA, protecting the hamsters from liver damage by *Eh* and favoring the mice a rapid resolution.

PM221 Antibacterial Activity of a Topoisomerase I Inhibitor versus Fluoroquinolones in Streptococcus pneumoniae biofilms

Myriam Valenzuela[1], María José Ferrándiz[2], Adela G. de la Campa[2], Maria Teresa Garcia Esteban[1]

[1] Universidad Complutense, Facultad de C.C. Biológicas, Genética, Fisiología y Microbiología, Ciudad Universitaria, Madrid, Spain
[2]Instituto de Salud Carlos III, Centro Nacional de Microbiología, Unidad de Genética Bacteriana, Majadahonda, Madrid, Spain

Background: *Streptococcus pneumoniae* is cause of community-acquired pneumonia, meningitis, otitis media, sinusitis and conjunctivitis. The increase of non-vaccine serotypes, nonencapsulated strains and fluoroquinolone-resistant isolates are emerging problems. Biofilm formation is associated with the persistence of disease and antibiotic resistance. Seconeolistine (SCN) is a new lead compound for antibiotic development that inhibits the growth of Streptococcus pneumoniae by inhibiting their DNA topoisomerase I enzyme.

Objectives: To compared the in vitro antibacterial activity of SCN with that of two fluoroquinolones (FQ), levofloxacin (LVX) and moxifloxacin (MFX), in biofilms formed by 2 isogenic pneumococcal strains: the susceptible R6 and the FQ-resistant T2.

Methods: *Streptococcus pneumoniae* R6 biofilms were analyzed after their treatment and during the postantibiotic phase. Growth kinetics of sessile bacteria forming the biofilm (viable counting), quantification of bacteria in the biofilm (staining with crystal violet) and confocal structure of the biofilm (confocal microscopy) were analyzed

Results: SCN was the drug with faster and higher bactericidal activity. Both FQs and SCN induced PAE in sessile bacteria forming biofilms at 2.5-5 $\times$ MIC, ranging between 0.99-3.32 h (LVX), 0.89-1.91 h (MFX) and 0.84-2.31 h (SCN). Longer PAE was induced by SCN than by fluoroquinolones. The inhibitory activity and the PAE induced by SCN were similar in both strains. However, they were lower in the resistant strain T2 treated with the FQs. PAE decreases of up 1.7 (LVX) or 1.4-fold (MFX) during biofilm formation. Therefore, Topoisomerase I Inhibitors could be an alternative to the treatment of pneumococci biofilms, especially against fluoroquinolone-resistant strains

PM222 Molecular mechanisms of colistin resistance among pandrug-resistant Acinetobacter baumannii isolates from Chile

Andrés Opazo-Capurro[1], Iván San Martín[1], Paulina González[1], Helia Bello-Toledo[1], Celia Lima[1], Gerardo González-Rocha[1]

[1]*Universidad de Concepción, Microbiology, Concepción, Chile*

Background: Carbapenem-resistant *Acinetobacter baumannii* (CRAB) has been recently deemed as a critical-priority pathogen by the World Health Organisation (WHO). Colistin (CST) represents the last resort to treat serious infections caused by CRAB, thus strengthening the surveillance of colistin is urgently needed. In Chile, limited data concerning resistance to CST in this pathogen are available, and a few colistin and pandrug-resistant (PDR) isolates have been detected lately.

Objectives: To identify the main mechanisms of CST-resistance in seven non-repetitive PDR-*Acinetobacter baumannii* isolates collected from nosocomial infections in Chilean hospitals between 2008 and 2014.

Methods: Seven *A. baumannii* isolates collected in Chilean hospitals were analysed. Antibiotic susceptibility tests were performed following the CLSI's guidelines. Genetic relatedness was determined by pulsed-field gel electrophoresis (PFGE) and by single-locus $bla_{\text{OXA-51-like}}$ sequence-based typing (SBT). CST-resistance mechanisms were analysed by the PCR amplification and sequencing of *lpxA*, *lpxC* and *lpxD* genes. Additionally, expression levels of the *pmrAB* system were measured by RT-qPCR.

Results: Two genetic clusters were identified by PFGE, including isolates recovered from the same hospitals, while the lineages associated with OXA-67 and -219 variants predominate. The minimum-inhibitory concentrations (MICs) to CST ranged from 4 to ≥512 µg/ml. We detected single nucleotide polymorphisms on the *lpx* genes not previously described in the PDR isolates. Moreover, the *pmrAB* system was over-expressed in isolates with higher CST MIC values.

PM223 Presence of wolbachia pipientis in trichuris sp. from macaca sylvanus

Julia Rivero Fernández[1], Rocío Callejón Fernández[1], Antonio Zurita Carrasco[1], Ángela García Sánchez[1], Cristina Cutillas Barrios[1]

[1]*University of Seville, Microbiology and Parasitology, Seville, Spain*

Background: Trichuriasis is the third most common roundworm infection of humans. Whipworm disease is a common intestinal infection with a worldwide distribution although it is more prevalent and severe in tropical and subtropical regions. Trichuriasis are being controlled through mass administration of antibiotics and other drugs.

The possibility of new strategies of control of parasite diseases have emerged with the discovery of the presence of the endosymbiont *Wolbachia* in arthropods and nematodes. The obligate endosymbiont has been recognized as a target for anti-filarial nematode chemotherapy as evidenced by the loss of worm fertility and viability upon antibiotic treatment in an extensive series of human trials.

Objectives: The aim of this work was to detect the presence of endosymbionts in *Trichuris* sp. isolated from captive macaco.

Methods: Molecular detection of *Wolbachia* sp. in *Trichuris* sp. from *Macaca sylvanus* were performed by Polymerase Chain Reaction (PCR) using the 16S ribosomal RNA partial gene of this endosymbiont as molecular target.

Results: 23 adult (11 males + 12 females) *Trichuris* sp. were assayed for the presence of *Wolbachia* sp. A 13 % of the population assayed were positive (1 male and 2 females). The posterior sequencing confirmed the specific identification as *Wolbachia pipientis*. This is the first report of the presence of this bacterium in *Trichuris* sp. Over the next several years, advanced application of molecular, biochemical and cytological techniques will greatly enhance the understanding of the biology and evolution of *Wolbachia* endosymbionts and enable application of that knowledge toward human parasite disease control.

PM224 Characterization of biofilm formation and antibiotic susceptibility in a clinical Acinetobacter baumannii strain

Paula Da Cunda[1], Victoria Iribarnegaray[1], Romina Papa[2], Ines Bado[2], María José González[1], Pablo Zunino[1], Rafael Vignoli[2], Paola Scavone[1]

[1]*Instituto de Investigaciones BiolOgicas Clemente Estable, Departamento de Microbiología, MONTEVIDEO, Uruguay*
[2]*Instituto de Higiene, Departamento de Bacteriologia y Virologia, Montevideo, Uruguay*

Background: *Acinetobacter baumannii* is an emerging opportunistic pathogen, one of the main microorganisms responsible for outbreaks in nosocomial infections worldwide. Its pathogenicity is mainly due to its resistance to multiple antibiotics and to its ability to form biofilms on abiotic surfaces

Objectives: The objective of this study, was to characterize the stages of biofilm formation of *A. baumannii* on a static biofilm model and to evaluate the antimicrobial susceptibility in planktonic and biofilm cells.

Methods: A clinical isolate was characterized for classical antimicrobial susceptibility test and in the biofilm after 48 h of formation. The capability to form biofilm was assayed first throught crystal violet technique in microtitre plates and also over glass coverslip. At different time point, the slides were subjected to live/dead staining and visualized using Confocal Microscopy. Image analyses were performed in order to evalute the different stages of the biofilm through morphotopological descriptors.

Results: The strain was only sensitive to gentamycin and colistin in planktonic cells but in biofilm the antibiotics were unable to kill the bacteria and to erradicate the biofilm. The evolution of the biofilm starts as fast as 12 hours and the irreversible adhesion and macrocolonies appears at 16 h. After maturation around 17-21 h we were able to determine that dispersion occurs at 24, 28 and 32 h.

PM225 Vancomycin-resistant Enterococcus encoded genes required for gut colonization

Alejandra Flor Duro[1], Javier Pons[1], Sandrine Isaac[1;2], Vincent de Maat[3], Willem van Schaik[3;4], Carles Ubeda[1;5]

[1]FISABIO, Genómica y Salud, Valencia, Spain
[2]EPFL, Ecole Polytechnique Fédérale de Lausanne, Laboratory of Molecular Microbiology, Lausanne, Switzerland
[3]University Medical Center Utrecht, Department of Medical Microbiology, Utrecht, Netherlands
[4]University of Birmingham, Institute of Microbiology and Infection, United Kingdom
[5]CIBER - Center for Biomedical Research Network, Epidemiología y Salud Pública, Spain

Background: Multidrug-resistant bacteria, such as vancomycin-Resistant-Enterococcus (VRE), are an increasing problem in hospitalized patients. Infections caused by VRE generally begin by colonization of the intestinal tract after the microbiota has been disrupted with antibiotics. However, very little is known about the VRE-encoded genes required for gut colonization.

Objectives: Identification of VRE encoded genes required for colonization of the intestinal tract.

Methods: We have utilized a mouse model of infection and a previously described methodology (Zhang et al., 2017, BMC Genomics) based on the generation of a transposon mutant library coupled with high-throughput sequencing.

Results: Our analysis have identified 50 genes whose disruption significantly reduces (>2 fold) VRE gut colonization in the large intestine. The genes that most affected VRE gut colonization encoded for proteins functionally annotated as: phosphotransferase system (PTS) for mannose acquisition, N-acetylmuramic acid 6-phosphate etherase, Lac-I family transcriptional regulator, bifunctional acetaldehyde-CoA/alcohol dehydrogenase, DsbA family protein, Fe-S cluster and a Phosphate ABC transporter ATP-binding protein. The specific role of these genes in gut colonization have been confirmed through targeted mutagenesis and competition experiments against a wild type strain. In addition, metatranscriptomic analysis identified another set of VRE genes encoding for a PTS ascorbate operon and a PTS sorbitol transporter, whose expression is highly overexpressed *in vivo*. Although the molecular mechanisms by which these genes affect gut colonization are not completely understood, our results have pinpointed several VRE encoded proteins, whose inhibition could.

PM226 Structure and function of the Campylobacter sp. type 6 secretion system

Kasia Radomska[1], Martin Pilhofer[1]

[1]*ETH Zürich, Department of Biology, Zürich, Switzerland*

Background: Pathogenic bacteria utilize a variety of secretion systems to manipulate their hosts. *Campylobacter* sp., the dominant cause of bacterial food-borne diseases, lack the type 3 and 4 secretion systems that are used by other enteropathogens to deliver virulence factors. A number of *Campylobacter* isolates however encode the type 6 secretion system (T6SS), which involvement in host-pathogen interactions remains unclear.

Objectives: We aim to determine the structure of the *Campylobacter* T6SS in its cellular context and to evaluate the functional role of the T6SS in the invasion process.

Methods: Western-blotting was used to detect the Hcp protein secretion to the extracellular milieu, a hallmark of the T6SS activity. The T6SS sheaths were isolated from *C.jejuni* and *C.coli* and visualized by negative stain transmission electron microscopy (TEM). Extended and contracted T6SS structures were imaged *in situ* by electron-cryo-tomography (ECT) of whole bacterial cells. The engagement of the T6SS in the host-pathogen interaction was verified using infection assays combined with confocal microscopy and cryo-focused ion beam milling of infected eukaryotic cells, followed by ECT.

Results: Western-blotting with Hcp-specific sera and negative stain TEM of the sheath preparations confirmed that *C.jejuni* and *C.coli* express and assemble a functional T6SS. The low-resolution *in situ* structure of a contracted T6SS sheath was determined by ECT and subtomogram averaging. Preliminary data suggest that T6SS-positive-strains have an advantage over T6SS-negative-strains in the invasion of human cell lines. Overall, presented data provide a groundwork for the determination of a high-resolution structure and function of the *Campylobacter* sp. T6SS.

PM227 The role of saoABC operon in intracellular survival and persister formation in Staphylococcus aureus

Anna Madry[1], Rafal Zagorski-Przybylo[1], Benedykt Wladyka[1], Michal Bukowski[1]

[1]*Jagiellonian University, Department of Analytical Biochemistry, Faculty of Biochemistry, Biophysics and Biotechnology, Krakow, Poland*

Background: The global gene transcription regulatory network modulates diverse interactions between bacterial and host cells, including those leading to persister formation, which are an important cause of chronic or recurrent infections. However, next to regulatory systems clearly demonstrated to play a role in this phenomenon in *Staphylococcus aureus*, there are still ones which may fit into the bigger picture as well.
Objectives: The aim of the study is to investigate the role of staphylococcal *saoABC* operon in persister formation and survival of *S. aureus* internalised into host cells as well as to identify factors involved into transcriptional regulation of the operon genes.
Methods: The applied methods included null mutant generation by allelic replacement, gene disruption by group II introns, analysis of survival of bacteria internalised to human cells *in vitro*, assessment of the ability to form persisters as well as pull-down assays for determination of proteins binding conserved regulatory sequences of *saoABC* operon.
Results: A clear growth rate decrease in nutrient-limiting conditions as well as a significant change in persister formation, reversible by gene supplementation on a plasmid, was observed for Δ*saoB* mutant next to decreased intracellular survival of Δ*saoC* mutant in human fibroblasts. Moreover, binding of different protein factors to yet uncharacterised regulatory sequences present in *saoABC* operon promoters was demonstrated.
The project was partially financed by funds granted to MB by the National Science Centre in Poland No. 2016/21/D/NZ1/00273.

PM228 Zebrafish model for fmbrial adhesin study in atypical enteropathogenic Escherichia coli

Danielle Munhoz[1], Amanda Richards[2], Fernando Martins[1], Matthew Mulvey[2], Roxane Piazza[1]

[1]*Butantan Institute, Bacteriology Laboratory, Sao Paulo, Brazil*
[2]*University of Utah, Division of Microbiology and Immunology, Department of Pathology,, Salt Lake City, United States*

Background: The colonization of atypical EPEC into host cells is a multifactorial process, thus different adhesins act simultaneously or at different stages. The *E. coli* common pilus (ECP) plays a role in bacterial adherence *in vitro*. But, no reports of its function in intestinal colonization through *in vivo* assays. The zebrafish (*Danio rerio*) model has as advantages: its small size, transparency during development, short generation time, genomic similarity with mammals and well-developed immune system.

Objectives: To evaluate the role of ECP in aEPEC adhesion in an *in vivo* model.

Methods: We investigated *ecp* operon genes presence by PCR and ECP production by immunofluorescence assays. The zebrafish model was selected for intestinal colonization assays with 96h.p.f larvae for 72 hours of infection.

Results: 96% of aEPEC strains presented *ecp* complete operon, but only 57% were ECP producers. In the intestinal colonization assays, BA1250D*ecpA* (major pilin knockout) showed a significant reduction in adhesion compared to wild type strain. However, BA1250D*operon* (full operon knockout) had no decrease of adhesion. Besides, in imaging assays BA1250D*operon:mCherry* showed adhesion variation at the site compared to WT only in the initial 24h.p.i., which was reverted to the WT pattern after 48-72h.p.i. These findings show that aEPEC is able to express different adherence factors to undergo the absence of ECP. In this sense, we established an *in vivo* model in zebrafish as a tool for the study of fimbrial adhesins and aEPEC infection.

PM229 Exposure to the innate immune system of Galleria mellonella alters polymyxin resistance in Enterobacter cloacae complex clinical isolates

Michael C. Gilmore[1], Inmaculada Garcia-Romero[1], Toby Bartholomew[1], Miguel A. Valvano[1]

[1]*Centre for Experimental Medicine at Queen's University, Belfast, United Kingdom*

Background: The polymyxins have recently been revived for the treatment of multidrug-resistant Gram-negative pathogens, but unfortunately, resistant bacteria are appearing at an alarming rate. Understanding the molecular mechanism involved in the resistance to polymyxin is of great importance; however, some bacteria show a heteroresistance phenotype that complicates the study of antibiotic resistance.

Objectives: This study explores heteroresistance to the polymyxins *in vivo* using representative isolates of the *Enterobacter cloacae* complex when the bacteria are exposed to the immune system of *Galleria mellonella* larvae.

Methods: *G. mellonella* larvae were infected with *E. cloacae* complex clinical isolates. After 24 hours of incubation, haemolymph was extracted and plated on Mueller-Hinton agar with doubling polymyxin B (PmB) concentration to isolate resistant subpopulations. Lipopolysaccharide (LPS) and Lipid A of inoculated isolate and the bacteria isolated upon infection were compared by SDS-PAGE and MALDI-TOF mass spectrometry, respectively. The expression of genes typically involved in PmB resistance was also compared by RT-qPCR.

Results: Infection in *G. mellonella* increased the overall PmB resistance in the majority of the strains. Mass spectra for the post-infection resistant subpopulations showed ion peaks which were not present in those obtained from the inoculated isolates. These peaks corresponded with m/z values consistent with the addition of L-aminoarabinose (L-Ara4N) to the lipid A, a modification associate with increased PmB resistance. In addition, the postinfection isolates demonstrated increased expression of genes involved in L-Ara4N biosynthesis, oxidative stress and efflux pumps. We conclude that the host selects for PmB resistant isolates upon infection.

PM230 Two Pseudomonas aeruginosa epidemic lineages. What is the difference?

Olga Voronina[1], Marina Kunda[1], Ekaterina Aksenova[1], Natalia Sharapova[1], Andrey Semenov[1], Natalia Ryzhova[1], Naylia Zigangirova[1], Alexander Gintsburg[1]

[1]N. F. Gamaleya National Research Center for Epidemiology and Microbiology, Ministry of Health of Russia, Moscow, Russian Federation

Background: Pseudomonas aeruginosa, causing nosocomial infections, form stable epidemic lineages, which change each other over time. P. aeruginosa ST (sequence type) 549 represents old epidemic lineage of clonal complex PA01. P. aeruginosa ST 2592, recently emerged in Russian hospitals, forms new epidemic lineage.

Objectives: The aim of this study was comparative analysis of virulence factors in strains representing two epidemic lineages for characterizing the adaptation potential of P. aeruginosa to hospital conditions.

Methods: Genomes and transcriptomes of clinical strains P. aeruginosa GIMC5015:PAKB6 (PAKB6, bronchoalveolar lavage, ST549*)* and P. aeruginosa GIMC5016:PA1840 (PA1840, burn wound*,* ST2592*)* were sequenced by Illumina platform. Data were analyzed and assembled by CLC v. 9.5.2; specialize programs and registered with Accession Numbers CP034429 and CP034430.

Results: Beta-lactamase genes compose the important part of cell resistome. PA1840 has additional beta-lactamase of class A and D with high level of transcriptional activity. Polimyxyn resistanse operon pmrHFIJKLM-udg and its regulatory systems (pmrAB, phoPQ) were similar in both strains with the exception of 4 amino acid substitutions in histidine kinase PmrB. However the level of operon transcriptional activity was higher in PA1840 suggesting polymyxin resistance of this strain. In the group of porins OprD had significant changes in the structure and low transcriptional activity in 1840 indicating difference in imipenem resistance. A set of pyocins was more divers in PAKB6 with high transcriptional level of the most of them.

Conclusion New P. aeruginosa epidemic lineage has advantage in resistance to medicine, and old epidemic lineage has competitive success in microbial community.

PM231 T3SS2 senses intracellular potassium ion to translocate T3SS2 effectors efficiently

Toshio Kodama[1], Sarunporn Tandhavanant[1;2], Shigeaki Matsuda[1], Hirotaka Hiyoshi[1;3], Tetsuya Iida[1]

[1]*RIMD, Osaka University, Suita, Osaka, Japan*
[2]*Faculty of Tropical Medicine, Mahidol University, Thailand*
[3]*School of Medicine, University of California, United States*

Background: Many Gram-negative symbionts and pathogens utilize a type III secretion system (T3SS) for their benefit and/or pathogenesis. The T3SS is a sophisticated secretion system for direct delivery of effectors into the host cell cytosol. The injected effectors then modify host cell functions. For effective delivery of effector proteins, bacteria need both to recognize host cell attachment and to switch the type of secreted proteins. *Vibrio parahaemolyticus* is a causative agent of food poisoning worldwide. T3SS2, which is encoded in an 80-kb pathogenicity island, is considered to be a major virulence factor for enteropathogenicity. However, secretory regulations of T3SS2 are largely unknown.

Objectives: In this study, we tried to identify a gatekeeper protein and a host factor, which control T3SS2 secretion.

Methods: *V. parahaemolyticus* strain RIMD2210633 (KP-positive, serotype O3:K6) was used for parent strain.

Results: VgpA and VgpB were identified as gatekeeper protein of T3SS2. In the absence of these gatekeepers, effector secretion was activated, but translocator secretion was abolished, causing the loss of virulence. We found that K^+ concentrations, which are high inside the host cell but low outside, are a key factor for VgpA- and VgpB-mediated secretion switching. Thus, T3SS2 senses the high intracellular concentration of K+ of the host cell so that T3SS2 effectors can be effectively injected.

PM233 Impact of Dermanyssus gallinae infestation on persistent outbreaks of fowl typhoid in commercial layer farms

Min-Su Kang[1], Jin-Hyun Kim[1], Ok-Mi Jeong[1], Su-Jeong Kim[1], Ji-Yeon Jeong[1], Hye-Jin Lee[1], Dong-Wan Kim[1], Jong-Ung Yoon[2], Yong-Kuk Kwon[1]

[1]Animal and Plant Quarantine Agency, Avian Disease Research Division, Gimcheon, Korea, Rep. of South
[2]Biogenoci Co., Ltd., Suwon-si, Korea, Rep. of South

Background: Fowl typhoid still occurs in commercial layer chickens causing a significant economic loss in Korea although it has rapidly decreased since the early 2000s. There is growing concern about the emergence of new pathogenic strains of the causative agent, *Salmonella* Gallinarum, able to overcome vaccine immunity. It has been also suspected that the poultry red mite, *Dermanyssus gallinae*, which is commonly found in layer farms, is an important cause of the recurrence of fowl typhoid in the farms.

Objectives: This study was to examine any change in virulence of recent isolates of *Salmonella* Gallinarum obtained from layer farms and to estimate the potential of the disease transmission by *D. gallinae* in the farms.

Methods: Clinical and environmental samples and mites collected from layer farms affected by fowl typhoid were tested for *Salmonella* Gallinarum. Isolates were characterized by biochemical tests and genotypic analyses. Their virulence was tested by infection experiments with the chicken cell line and young chickens. We also analyzed the epidemiological relationship between the infestation by *D. gallinae* and the recurrence of fowl typhoid in the farms.

Results: There was no evidence of increase in virulence of recent isolates of *Salmonella* Gallinarum. Epidemiological analysis indicated that farm environmental factors, including the infestation by *D. gallinae*, and insufficient vaccine immunity were significantly implicated in outbreaks of fowl typhoid in layer farms. It was also demonstrated that the horizontal transmission and environmental persistence of *Salmonella* Gallinarum in layer farms could be mediated by *D. gallinae*.

PM234 Using fourier-transform infrared spectroscopy to analyze a clonal outbreak of extended-spectrum beta-lactamase-producing klebsiella pneumoniae

Nadya Rakovitsky[1], Jonathan Lellouche[1], Sammy Frenk[1], Mitchell Schwaber[1;2], David Schwartz[1], Hadas Kon[1], Yehuda Carmeli[1;2], Ester Solter[1], Svetlana Paikin[3], Regev Cohen[3;4]

[1]*National Institute for Antibiotic Resistance and Infection Control, Tel Aviv, Israel*
[2]*Sackler Faculty of Medicine, Tel-Aviv University, Tel Aviv, Israel*
[3] *Infectious Diseases Unit, Sanz Medical Center, Laniado Hospital, Netanya, Israel*
[4]*The Ruth and Bruce Rappaport Faculty of Medicine, Technion-Israel Institute of Technology, Haifa, Israel*

Background: Microbial typing methods are used to support outbreak investigation. PFGE and PCR-based methods (e.g. Box-PCR) are labour-intensive, time-consuming and occasionaly not descriminatory. Whole genome sequences (WGS) become the gold-standard, however, it is expensive, time-consuming, and requires expertise. A novel method for typing based on Fourier-transform infrared (FTIR) spectroscopy that measures the vibrations of carbohydrate in the bacterial cell-wall was recently introduced.

Objectives: To compare FTIR to BOX-PCR and WGS for bacterial typing in an outbreak of ESBL-producing *K. pneumoniae* in a neonatal intensive-care-unit (NICU).

Methods: ESBL-producing *K. pneumoniae* isolates (n=19) collected during a NICU outbreak (June-August 2017). Source of isolates were clinical cultures (n=4), mother birth canal (n=1), rectal swabs (n=13), and the environment (n=1). Isolates were characterized by: 1. FTIR using an IR Biotyper (Bruker) followed by hierarchical cluster analysis using Opius 7.5 software. 2. BOX-PCR followed by cluster analysis using an unweighted pair group method with arithmetic mean (UPGMA). 3. WGS by MiSeq (Illumina) and scaffolds were assembled *de novo* using SPAdes. Data analyzed using the online CGE tool.

Results: BOX-PCR classified the 19 isolates into major clone (n=10), secondary clone (n=4) and single clones (n=5). WGS, classified one of the secondary clone isolated as a singelton . FTIR, was easy and rapid to perform, and was in complete agreement with the WGS. Real world experience with FTIR, showed it is an easy to apply and had similar performance as WGS in this NICU outbreak.

PM235 Comparative genomics and transcriptomics of Salmonella clinical isolates representing different cell line infecting phenotypes

Rafal Kolenda[1;2], Michal Burdukiewicz[3], Aamir Ali[4], Istvan Szabo[5], Adrianna Aleksandrowicz[1;6], Jörg Nitschke[2], Alexander Böhm[2], Josefine Bartholdson Scott[7], Dereck Pickard[7], Gordon Dougan[7], Peter Schierack[2]

[1]*Wrocław University of Environmental and Life Sciences, Department of Biochemistry and Molecular Biology, Wrocław, Poland*
[2]*Brandenburg University of Technology Cottbus–Senftenberg, Senftenberg, Germany*
[3]*Warsaw University of Technology, Warszawa, Poland*
[4]*NIBGE, Faisalabad, Pakistan*
[5]*National Salmonella Reference Laboratory, (BfR), Berlin, Germany*
[6]*University of Wrocław, Wrocław, Poland*
[7]*Wellcome Sanger Institute Sulston Building, Hinxton, United Kingdom*

Background: Adhesion and invasion to various epithelial cells are among the first steps during the pathogenesis of Salmonella infections. Salmonella has evolved various strategies to adhere and invade its host cells. Moreover, different serovars have developed specific infection phenotypes.

Objectives: Our objective was to develop an automated microscopy-based system for bacterial infection assays. Next, we aimed to identify genes responsible for different infecting phenotypes in clinical isolates of Salmonella.

Methods: 127 Salmonella isolates from five serovars of human, cattle, swine and chicken origin were transformed with the GFP plasmid and used in infection assays on IPEC-J2, Caco-2 and CHIC-8E11 cell lines. Adhering/infecting bacteria were counted after 1 h and 4 h of infection with the automated VideoScan microscope. The genomes of 30 isolates with highest and lowest median adhesion/infection values and RNAs from 4 S.Typhimurium and 4 S.Enteritidis isolates were sequenced.

Results: In screening of 127 Salmonella isolates, the median infection value was found highest for CHIC-8E11 and lowest for IPEC-J2 cells. Among all tested Salmonella serovars on cell lines, S.Gallinarum showed lowest infection value on CHIC-8E11 and S.Choleraesuis had the highest infection value on Caco-2. Overall, S.Gallinarum displayed the lowest infection values regardless on the incubation time. Comparative genome analysis revealed differences (SNPs, deletions) in following genes: dksA, nmpC, yidR, sanA, rfaL, sirA, aroA, lrhA, rcsD, ompW, mpl, rtsA, wza. Analysis of transcriptomes showed disparate expression profiles of genes associated with invasion of cells (e.g. sopA, sopE, sipA-sipD, invA-invE) in strains with different infection phenotypes.

PM236 A small colony variant of Escherichia coli has increased susceptibility to co-trimoxazole, reduced fitness and impaired ability to form biofilms

Alasdair Hubbard[1;2], Adam Roberts[1;2]

[1]*Liverpool School of Tropical Medicine, Parasitology, Liverpool, United Kingdom*
[2]*Liverpool School of Tropical Medicine, Centre for Drugs and Diagnostics, Liverpool, United Kingdom*

Background: Small colony variants (SCVs) of *Staphylococcus* are common and well reported. SCVs of *Escherichia coli*, however, are rarer although they can still be etiological agents of infections and are therefore are clinically relevant.

Objectives: We identified a SCV of *E. coli* following *in vitro* selection of antimicrobial resistant mutants using sub-inhibitory concentrations of amoxicillin-clavulanic acid (AMC) and gentamicin. We characterised the antimicrobial resistance profile and biofilm production of the SCV and identified the mutation responsible for the SCV and the fitness effect of this mutation.

Methods: The SCV was resistant to both AMC and gentamicin, however; it was more susceptible to co-trimoxazole compared to the two ancestral strains. Assessment of competitive fitness and biofilm production of the SCV compared to the ancestor isolates found that it incurred a significant fitness cost and impaired biofilm formation. Addition of 20 μg/ml hemin to agar resulted in reversion of the SCV to an equivalent size of the ancestral isolates, however, addition to liquid media did not result in improved fitness or biofilm formation comparable to the ancestor. Whole genome sequencing identified a novel, stable, single nucleotide polymorphism (SNP) within *hemA*.

Results: A SNP in *hemA* is responsible for a SCV of *E. coli* which is more sensitive to co-trimoxazole, has impaired biofilm production and is competitively unfit. The presence of hemin reverts morphology to equivalent size of the ancestor but does not affect biofilm formation or fitness, potentially due to inefficient biosynthesis of heme.

PM237 Mice infection with Candida glabrata biofilm cells: inflammatory cell recruitment and antifungal treatment efficacy

Celia Fortuna Rodrigues[1;2], Alexandra Correia[3], Mariana Henriques[2], Manuel Vilanova[3]

[1]*LEPABE, Chemical Engineering, Porto, Portugal*
[2]*University of Minho, Biological Engineering, Braga, Portugal*
[3]*University of Porto, Instituto de Ciências Biomédicas Abel Salazar, Porto, Portugal*

Background: *Candida glabrata* is one of the most widespread *Candida* spp. associated to systemic candidiasis. This species is particularly critical in hospitalized, catheterized and immunosuppressed patients, due to a high drug resistance, specially to the azoles, but also to the ability to rapidly develop echinocandin resistance.

Objectives: The goal of this work was to simulate a systemic infection exclusively derived from *C. glabrata* biofilm cells and to evaluate the effectiveness of two echinocandins – caspofungin (Csf) and micafungin (Mcf) - in its treatment. The host-pathogen response was also studied, by analyzing the inflammatory cell recruitment.

Methods: CD1 mice were infected exclusively with 48 h-biofilm cells of *C. glabrata* and then treated with Csf or Mcf. After 72h, the efficacy of each drug was evaluated assessing organ fungal burden through CFU counting. Moreover, the immune cell recruitment into target organs was evaluated by flow cytometry and histopathology analysis.

Results: Fungal burden was higher in the liver than in the kidneys. Nevertheless, none of the drugs was effective in eradicating completely the infection. At the evaluated time point, flow cytometry analysis, showed a predominant mononuclear response in the spleen, which was also evident in liver and kidneys of the infected mice, as observed by the histopathology analysis. Together, these observations confirmed *C. glabrata* as a low inflammatory species and indicated that two-dose treatment with Csf and Mcf do not have a significant impact on liver and kidney fungal burden, or recruited inflammatory infiltrate, when mice are i.v. infected with *C. glabrata* biofilm-grown cells.

PM238 Inflammation associated ethanolamine facilitates infection by Crohn's disease-linked adherent-invasive Escherichia coli

Michael Ormsby[1], Michael Logan[2], Sile Johnson[1], Anne McIntosh[1], Ghaith Fallata[1], Rodanthi Papadopoulou[3], Eleftheria Papachristou[3], Richard Hansen[4], Umer Ijaz[2], Richard Russell[4], Konstantinos Gerasimidis[3], Donal Wall[1]

[1]Institute of Infection, Immunity and Inflammation, College of Medical, Veterinary and Life Sciences, University of Glasgow, Bacteriology, Glasgow, United Kingdom
[2]School of Engineering, University of Glasgow, Glasgow, United Kingdom
[3]School of Medicine, College of Medical, Veterinary and Life Sciences, Human Nutrition, Glasgow, United Kingdom
[4]Hepatology and Nutrition, Royal Hospital for Children, Paediatric Gastroenterology, Glasgow, United Kingdom

Background: The predominance of specific bacteria within the Crohn's disease intestine remains poorly understood with little evidence uncovered to support a selective pressure underlying their presence. Intestinal ethanolamine is readily accessible during periods of intestinal inflammation, and enables pathogens to outcompete the host microbiota under such circumstances.

Objectives: We endevour to show that the intestinal short chain fatty acid propionic acid stimulates increased ethanolamine degradation by one such Crohn's disease associated pathogen, adherent-invasive Escherichia coli (AIEC).

Methods: Transmission electron microscopy (TEM); qPCR; gel-free proteomics; intracellular bacterial replication through tissue-culture; and microbial mRNA extraction and enrichment from faecal samples.

Results: Propionic acid exposure stimulates ethanolamine degradation. This degradation occurs within bacterial microcompartments that are subsequently excreted in outer membrane vesicles. Additionally ethanolamine, added extracellularly at concentrations comparable to those in the human intestine, is accessible to intracellular AIEC and stimulates significant increases in growth within macrophages. Finally, expression of the operon for ethanolamine degradation (*eut*) is increased in children with active Crohn's disease compared to healthy controls. After clinical remission was seen with exclusive enteral nutrition treatment, Crohn's disease patient's exhibit significantly reduced *eut* expression. Our data indicates a role for ethanolamine metabolism in facilitating AIEC colonization of the Crohn's disease intestine and warrants further study of its potential use as an indicator of inflammatory status in Crohn's disease.

PM239 The virulence-essential secretion system ESX-4 in M. abscessus is regulated by a small non coding RNA

Daniel Barkan[1]

[1]*Hebrew University, Veterinary Medicine, Rehovot, Israel*

Background: Small non-coding RNA (sRNA) play a regulatory role of virulence systems in many bacteria – however, their role in mycobacteria remains unknown.

Objectives: To understand the role played by sRNA in mycobacterial pathogenesis.

Methods: Using a novel, double antibiotic-selection transposition system, we created a comprehensive transposon-mutant library in the highly resistant pathogen *M. abscessus*.

Results: We identified three Tn-mutants with aberrant colony morphology. Two of them were shown to be defective in components of the virulence-essential ESX-4 secretion system, equivalent in function to the ESX-1 in Mtb (*esxU*, the secretion substrate, and *espI (Mabs_0630)*, a regulator). However, a third Tn-mutant was identified as an **overexpressor** of the sRNA B11, also present in Mtb. Whereas complementation of the *esxU* Tn-mutant with a functional *esxU* reverted the morphology back to "S", overexpression of B11 in wt (S) bacteria caused the same morphology change as an *esxU* deletion. Overexpression in other smooth mycobacteria, such as *M. kansasii*, caused the same morphology change.
In *M. abscessus*, overexpression of B11 prevented *esxU* secretion, without affecting the production - analogous to *phoP* control of ESAT-6 secretion in Mtb. When whole proteomics analysis of WT versus B11 overexpressor was performed, all the ESX-4 components were found to be regulated by B11.
In-silico binding-prediction models showed B11 had high affinity (9^{th} out of the whole genome) to Mabs_0628 RNA, the first gene in the *espI* operon, controlling ESX-4 system.
This is the first report of a pathogenesis-associated role of sRNA in mycobacteria.

PM240 Outcome of Staphylococcus aureus bacteremia in neutropenic patients with hematologic malignancy comparing to non-neutropenic patients without malignancy: A 10- year experience

Jun Hee Woo[1], Yong Pil Chong[1], Yang Soo Kim[1], Mee Soo Chang[2]

[1]University of Ulsan College of Medicine, Department of Infectious Diseases, Asan Medical Center, Seoul, Korea, Rep. of South
[2]Seoul National University College of Medicine, Department of Pathology, Seoul National University Boramae Hospital, Seoul, Korea, Rep. of South

Background: *Staphylococcus aureus* bacteremia (SAB) is one of the most serious bacterial infections. It may lead to worse outcomes in patients with prolonged severe neutropenia. However, there are limited data on clinical outcomes of SAB in neutropenic patients with hematologic malignancy(NH).

Objectives: To assess prognosis of SAB in NH compared to that in non-neutropenic patients without malignancy(NW) or neutropenic solid tumor patients(NS).

Methods: We evaluated clinical outcomes of SAB in NH, in NS, and in NW , using a prospective cohort of SAB patients at a tertiary-care hospital from Jul 2008 to Jun 2017.

Results: : Our cohort consisted of 2,613 patients ; 64 (2.4%) were NH, 24 (0.9%) were NS, and 847 (32.4%) were NW. Nosocomial acquisition and primary bacteremia were significantly more frequent in NH than in other two groups. There were no significant differences in incidence of metastatic infections among 3 groups (17.2% , 16.7% vs. 20%). Although the removal of eradicable focus was less frequently performed in NH, crude mortality of NH was not higher than that of other two groups. In particular, NH showed significantly lower 12-week mortality than NS (15.6% vs 45.8% p = 0.003), but not significantly lower than NW(19.8%). The independent risk factors for mortality were age, Charlson comorbidity score, longer duration (>3 days) of bacteremia, and presence of metastatic infection In multivariable analysis . In conclusion the findings suggest that clinical outcomes of SAB in NH are not significantly different from those in NW.

PM241 Genetic diversity and drug resistant of TB in Oaxaca, Mexico

Yuko Nakamura-Lopez[1;2], Lucia Martinez-Martinez[3], Perla Martinez[3;4]

[1]*Servicios de Salud de Oaxaca, Dirección de Prevención y Promoción a la Salud, Unidad de Epidemiología, Oaxaca, Mexico*
[2]*Universidad Autonoma Benito Juarez, Facultad de Medicina - Posgrado en Biomedicina Molecular, Oaxaca, Mexico*
[3]*Universidad Autonoma Benito Juarez, Facultad de Medicina - Laboratorio de Biologia Molecular, Oaxaca, Mexico*
[4]*Instituto Tecnologico de Oaxaca, División de Estudios de Posgrado e Investigación, Oaxaca, Mexico*

Background: Tuberculosis (TB) is a re-emerging health problem worldwide. In Mexico, information about genetic diversity of *Mycobacterium tuberculosis complex* (MTBc) is very limited especially in the state of Oaxaca, were incidence and mortality is higher than the national average. This could be associated with the diverse environments, cultures, economic lag, high migration rates and international tourism in the eight regions of the state.

Objectives: The aim of this work was to characterize patterns of genetic diversity and drug resistance in isolates of TB patients from Oaxaca, Mexico.

Methods: We analyze 463 clinical samples of patients with clinical suspicion of TB form Oaxaca, Mexico, by smear, culture and PCR. The positive samples to MTBc were subjected to genotyping by large sequence polymorphisms (LSPs), spoligotyping and 24-loci MIRU-VNTR typing methods. Comparing with the MIRU-VNTR plus database genotypic clade designations were done. Drug-resistance to first line drugs was performed by phenotypic and genotypic methods.

Results: MTBc were 21% of the samples and 16% were mycobacteria other than tuberculosis. We obtained 10 genotypes from which the sub-lineage Haarlem was the more frequent (31%), followed by East-African-Indian (EAI, 26%). EAI is the most ancestral sub-lineage and is rarely reported in Latin America, and 45.5% of them were resistant to at lest one drug. This study provides the first description of genetic diversity of MTBc in Oaxaca, Mexico. The finding of ancestral EAI as one of the principal genotypes contrast with other studies in Mexico.

PM242 New records of vector-borne pathogens in diferent species of fleas in the southwestern of europe

Antonio Zurita Carrasco[1], Maureen Laroche[2], Cristina Cutillas Barrios[1], Philippe Parola[2]

[1]*University of Seville, Microbiology and Parasitology, Seville, Spain*
[2]*University Hospital Institute Méditerranée Infection , Research unit for Vectors - Tropical and Mediterranean Infections (VITROME), Marseille, France*

Background: In the last years a high number of studies have reported the importance of fleas and human flea-borne infections. Some of these flea-borne human pathogens have re-emerged recently (e.g., *Bartonella henselae*, *Rickettsia felis*) suggesting that much remains to be learned about the potential role of fleas as disease vectors.

Objectives: The aim of this work was to detect and assess the presence and prevalence of several pathogen bacteria and symbionts in different flea species collected from domestic, peridomestic and wild animals from different geographical areas of Spain and France.

Methods: A total of 319 fleas belonging to eight different species were tested to assess the prevalence of genera and species of different bacteria using real-time quantitative and standard PCR techniques.

Results: Our results strongly support the role of *Wolbachia* spp. as a common endosymbiont of *Ctenocephalides felis* and *Pulex irritans*. On the other hand, *R. felis* was detected in *C. felis*, *Archaeopsylla erinacei* and *Ctenophthalmus baeticus boisseauorum*, whereas *Rickettsia typhi* was detected in *A. erinacei* by the first time. Furthermore, in this study we detected *Mycobacterium* spp. in three fleas from Spain belonging to three different species (*C. felis*, *P. irritans* and *A. erinacei*) and four different species of *Bartonella* infesting different fleas species. In conclusion, with this study we widened knowledge of the flea-borne pathogens present in the Southwestern of Europe. For medical and veterinary community it is critical to investigate the efficiency of different flea species in transmitting a variety of pathogens species.

PM243 Spread of antibiotic resistance genes by bacteriophage-like particles in human pathogens

Lorena Rodriguez-Rubio[1], Pedro Blanco-Picazo[1], Maryury Brown-Jaque[1], Ferran Navarro[2], Maite Muniesa[1]

[1]University of Barcelona, Genetics, Microbiology and Statistics, Barcelona, Spain
[2]Servei de Microbiologia, Hospital de la Santa Creu i Sant Pau, Institut d'Investigació Biomèdica Sant Pau, Barcelona, Spain

Background: Most environmental bacteria carry antibiotic resistance genes (ARGs). From these bacteria, ARGs are transferred to other bacteria by mobile genetic elements (plasmids, transposons or bacteriophages), so from an environmental source, ARGs could be transferred to clinical settings. Previous work from our laboratory confirmed the presence and abundance of ARGs in the phage fraction isolated from human feces. Preliminary observations points towards a sort of transduction mediated by phage-like particles produced by bacteria in high amounts, suggesting a mechanism used by bacteria to mobilize their genetic content.

Objectives: To describe the nature of the ARGs-containing phage particles detected in *Enterobacteriaceae* isolates and elucidate the mechanisms of generation that can lead to their spread and the emergence of new resistant strains.

Methods: Induction and isolation of particles carrying ARGs from *Enterobacteriaceae* isolates by mitomycin C and subsequent purification by centrifugation, filtration and CsCl gradient. Quantification of ARGs by qPCR. Direct observation of phage particles by electron microscopy. Detection of capsid proteins by SDS-PAGE and subsequent MALDI-TOF/TOF. Evaluation of the infectivity using fluorescence microscopy and flow cytometry.

Results: We confirmed the generation of ARG-containing particles after induction of several isolates. Electron micrographs showed tailed phage particles, corresponding to *Myoviridae* and *Siphoviridae* morphological types. Isolated particles showed densities of GC/ml > 10^7 for, at least, one of the ARGs studied. Proteomic analysis confirmed the presence of phage capsid proteins. Infectivity of *E. coli* WG5 strain (containing no prophages nor ARGs) by SyBr Gold labelled phage particles was confirmed.

PM244 Identification of the human receptor for the Sthapylococcus aureus lipoprotein Lpl and its role in cellular invasion

Paula Maria Tribelli[1;2], Arif Luqman[1], Friedrich Goetz[1]

[1]*Microbial Genetics, Interfaculty Institute of Microbiology and Infection Medicine Tübingen (IMIT), University of Tübingen, Tübingen, Germany*
[2]*IQUIBICEN-CONICET. Faculty of Exact and Natural Sciences. University of Buenos Aires, Biological chemistry, Buenos Aires, Argentina*

Background: *Staphylococcus aureus* is an opportunistic human pathogenic bacterial species that causes serious infections. Lipoproteins encoded in the lpl cluster triggers the invasion of *S. aureus* in nonprofessional phagocytic cells (NPPCs). *S. aureus* internalization by NPPCs is based on the adhesion to the host cell resulting in signal transduction and internalization of the bacteria. However, the receptor for Lpl protein in the host cells is still unknown.

Objectives: our objective is to determinate the receptor of Lpl1 in NPPCs.

Methods: Lpl1 protein was used as prototype of the Lpl proteins. We used soluble Lpl1 lacking the lipid signal peptide and a his-tag (Lpl1-his(-sp) to facilitate its purification from the cytosol of S. aureus using standard methods. Pull-down experiments were performed using Lpl1-his(-sp) as bait bound to Ni+2-column; HaCat cell lysate was used as a source of the receptor. Elution fractions were analyzed by SDS and stained using Coomasie blue. Visible bands in the experimental samples and absent in the control (without Lpl1) were cut and analyzed by Mass Spectrometry (MS).

Results: We successfully purified Lpl1-his(-sp) from S.aureus. In pull-down experiments we obtained three bands in which Lpl1-his(-sp) was interacting with a potential host cell receptor. The host proteins were analysed by MS analysis. We identified and discarded well-known cytosolic proteins regarding transcription, translation or metabolic functions. We selected two candidates and our analysis revealed that both proteins belong to a human chaperon family. The proteins are now under investigation.

PM245 vncr regulates pneumococcal capsule polysaccharide synthesis in a serotype-specific manner in the presence of serum lactoferrin

Prachetash Ghosh[1], masaud shah[2], subramaniyam ravichandran[3], hamid iqbal[1], dong-kwon rhee[1]

[1]*sungkyunkwan university, school of pharmacy, suwon, Korea, Rep. of South*
[2]*ajou university, department of molecular science and technology, suwon, Korea, Rep. of South*
[3]*sungkyunkwan university, school of medicine, suwon, Korea, Rep. of South*

Background: *Streptococcus pneumoniae* is the major etiologic agent of pneumonia, a leading factor of global health burden. Limitations in antibiotic therapy and vaccine efficacy draw sincere attention towards the progress of our understanding of host–pathogen interactions to improve alleviation strategies.

Objectives: Capsular polysaccharides (CPS), a major virulence factor in *S. pneumoniae*, becomes thickened during blood invasion than during asymptomatic nasopharyngeal colonization. As the CPS plays significant role in warding off the bactericidal host immune response, an elaborate knowledge of underlying mechanism regulating differential CPS expression is prerequisite to overcome the unmet medical needs.

Methods: We show how VncR, the response regulator of vancomycin resistance locus (*vncRS* operon), regulates CPS expression in *vncR* mutants in three serotype (type 2, 3, and 6B) backgrounds upon exposure to serum lactoferrin (LF). Comparative analysis of CPS levels by qRT-PCR and ELISA in the wild type (WT) of three serotypes and their isogenic *vncR* mutants after LF exposure revealed a serotype-specific alteration in CPS production. Consistently, VncR-mediated serotype-specific CPS production is correlated with pneumococcal virulence, *in vivo*. Electrophoretic mobility-shift assay (EMSA) and co-immunoprecipitation (co-IP) revealed an interaction between VncR and the *cps* promoter (*cpsp*) in the presence of serum. Moreover, *in silico* analysis uncovered this protein-DNA interaction, suggesting that VncR binds with the *cpsp*, and recognizes the serotype-specific significance of the tandem repeats in *cpsp*.

Results: These findings offer a thorough understanding of the regulation of differential serotype-specific CPS production mediated by VncR, which subsequently determines serotype-specific systemic virulence.

PM246 Vancomycin resistance among Enterococcus spp isolated from patients with urinary tract infections

Yinka Somorin[1], Nichola-Jane Weir[1], Maeve Higgins[2], Carmel Hughes[1], Deirdre Gilpin[1], Martin Crockard[2], Michael Tunney[1]

[1]School of Pharmacy, Queen's University Belfast, Belfast, United Kingdom
[2]Randox Laboratories Ltd, Crumlin, United Kingdom

Background: *Enterococcus* spp is an important pathogen in urinary tract infections (UTIs) and development of resistance to antibiotics in this pathogen has made treatment difficult. Vancomycin resistance in enterococci is of growing concern and the World Health Organization recently classified it as "high priority" for the development of new antibiotics.

Objectives: Since understanding the resistance profile of pathogens is important to determine optimal therapeutic options and effective treatment, this study investigated the incidence of vancomycin resistance among *Enterococcus* spp associated with UTI patients.

Methods: Clinical isolates of *Enterococcus* spp (n=109) obtained from the Belfast Health and Social Care Trust and the Northern Health and Social Care Trust in Northern Ireland were analyzed for susceptibility to vancomycin using Etest and detection of vancomycin resistance genes (*vanA* and *vanB*) by PCR.

Results: Etest results showed that all *Enterococcus faecalis* (n=102) were susceptible to vancomycin whereas 2/7 (28.6%) *E. faecium* were resistant to vancomycin. *VanA* was detected only in the two vancomycin-resistant *E. faecium* (UM130 and UM525) while *vanB* was detected in two *E. faecium* isolates (UM464 and UM511) which were both susceptible to vancomycin. Although vancomycin resistance among all enterococci in this study was low (1.8%), there was a high percentage among the few *E. faecium* isolates analyzed. Overall, this study showed that vancomycin resistance is present in the *E. faecium* isolates investigated. A larger and more detailed investigation of *E. faecium* is recommended in more healthcare settings to better understand VREs across Northern Ireland.

PM247 Cutibacterium acnes phylogenetic type IC and II isolated from patients with non-acne diseases exhibit high-level formation

Keisuke Nakase[1], Ren Midorikawa[1], Kento Yamasaki[1], Sae Aoki[1], Norihisa Noguchi[1]

[1]*Tokyo University of Pharmacy and Life Sciences, Department of Microbiology, School of Pharmacy, Tokyo, Japan*

Objectives: *Cutibacterium acnes,* a skin bacterium, causes surgical site infection (SSI). In addition, some *C. acnes* strains form biofilms on the surfaces of catheters and prosthetics. To investigate the characteristics of high-level biofilm formation by *C. acnes*, we studied 103 *C. acnes* strains isolated from outpatients and inpatients across 18 departments of university hospital. Biofilm quantification and phylogenetic typing were performed on these strains.

Results: Twenty-five (24.3%) and 17 *C. acnes* strains (16.5%) belonging to phylogenetic types IC and II, respectively, were isolated. These strains are rarely isolated from healthy skin or patients with acne. Assessment of biofilm formation indicated that 27.2% (28/103) of all strains belonged to high-level biofilm forming strains. By contrast, the strains isolated from acne patients showed significantly lower proportion of high-level biofilm forming strains (4.0%, P <0.05). Very few of IA1 strains (3.4% (1/29), P <0.05) which are dominant in healthy skin, while 76.0% (19/25) of type IC and 52.9% (9/17) of type II strains belonged in high-level formation strains. The high-level biofilm forming strains were frequently isolated from patients in emergency (55.6%), plastic surgery (50.0%), and orthopedic surgery (33.3%), and the strains of type IC and II accounted for a large portion of the strains isolated from the three departments (58.3-70.0%). No difference in phylogenetic type and proportion of high-level biofilm formation strains isolated from outpatients and inpatients was observed. Therefore, high-level biofilm forming strains of type IC and II were isolated in specific departments regardless of whether the patients were outpatients or inpatients.

PM248 Association of MLST sequence types and serotypes with pathotypes of Avian Pathogenic Escherichia coli (APEC)

Arnoud van Vliet[1], Jai Mehat[1], Huijin Long[1], Sarah Johns[1], Amanda Fivian-Hughes[1], Jonathan Betts[1], Martin Woodward[2], Roberto La Ragione[1]

[1]University of Surrey, School of Veterinary Medicine, Guildford, United Kingdom
[2]University of Reading, Department of Food and Nutrition Science, School of Chemistry, Reading, Reading, United Kingdom

Background: Avian pathogenic *Escherichia coli* (APEC) are extraintestinal pathogenic *E. coli* which cause colibacillosis in poultry. Despite their importance to the poultry industry, there is a lack of genetic and or serological markers that can distinguish commensal *E. coli* from APEC.

Objectives: To identify genetic markers specific for APEC.

Methods: Genome sequences from European APEC and public repositories were compared for sequence and serotypes.

Results: Phylogenetic analysis using core genome SNPs of 414 European APEC isolates showed multiple APEC clusters. The largest cluster was ST117 (n=66), followed by ST23 (n=48), ST95 (n=44) and ST140 (n=28). The ST117 cluster contained multiple O-serotypes including O78 and O24 with H4 flagellar serotype. In contrast, the ST23 cluster contained approximately equal numbers of O78:H4 and O78:H9 serotypes. The ST140 cluster contained O2:H5 isolates, and the ST95 cluster contained O1, O2 and O18 with H5 and H7. Analysis of 2,507 *E. coli* genomes from Genbank with source annotation available confirmed the links between APEC and these STs, while source data from an additional 1,359 isolates from Enterobase (Warwick) confirmed the link between avian sources and especially ST117, ST23 and ST140. The majority of the APECs in these clusters were positive for ≥5 virulence markers, a method used to distinguish APEC from commensal isolates. In conclusion, in this study we have shown that specific sequence types and serotypes are strongly associated with APEC, and these may allow for more accurate differentiation between commensals and APEC pathotypes, and future identification of virulence mechanisms and vaccine candidates.

PM249 Accurate prediction of anti-phagocytic activity of Vibrio vulnificus by measurement of bacterial adherence to hydrocarbons.

Takashige Kashimoto[1], Takehiro Kado[1], Kohei Yamazaki[1], Kaho Matsuda[1], Shunji Ueno[1]

[1]*Kitasato Univ., Veterinary Public Health, Towada, Japan*

Background: *Vibrio vulnificus* can cause necrotizing soft tissue infection via exposure through an open wound, and the incubation period in cases of wound infection is only about 16 h. These facts strongly suggest that mechanisms to evade innate immune cell phagocytosis are essential for its pathogenicity. Hydrophobic interaction is one of the binding mechanisms between bacteria and phagocytes. Several factors that maintain cell surface hydrophobicity (CSH) can contribute to anti-phagocytic activity.

Objectives: In this study, we tried to identify *V. vulnificus* genes involved in maintaining the CSH, in order to elucidate mechanisms of anti-phagocytic activity.

Methods & Results: We obtained 143 mutants that had lost their ability to proliferate in the host, using signature-tagged transposon basis mutagenesis (STM). The CSH of these mutants was measured by the bacterial adherence to hydrocarbons (BATH) assay. The CSH of only four mutants differed significantly from that of wild type (WT). Of these four mutants, *degS* mutant (*degS*::Tn) showed lesser anti-phagocytic activity than WT in the opsonophagocytosis assay, even though *degS*::Tn showed opaque-type colonies. Furthermore, survival times of mice subcutaneously inoculated with *degS*::Tn were prolonged. These facts indicated that the BATH assay is a more suitable method of analyzing the anti-phagocytic activity of *V. vulnificus* than the comparison of colony morphology.

PM250 Phenotypical and molecular assessment of the virulence potential of KPC3-producing Klebsiella pneumoniae ST392 clinical isolates

Danilo D'Apolito[1], Anna Paola Carreca[1], Giuseppina Di Mento[2], Nicola Cuscino[2], Giovanna Russelli[2], Gioacchin Iannolo[2], Salvatore Pasqua[1], Francesco Monaco[2], Francesca Cardinale[2], Pier Giulio Conaldi[2], Bruno Douradinha[3]

[1]Fondazione Ri.MED c/o IRCCS-ISMETT, Palermo, Italy
[2]IRCCS-ISMETT, Palermo, Italy
[3]Fondazione Ri.MED c/o IRCCS-ISMETT, Sviluppo Vaccini e Microbiologia Sperimentale, Palermo, Italy

Background: *Klebsiella pneumoniae* is a Gram-negative bacterium of clinical importance, which colonizes mucosal surfaces and, from there, gain access to other tissues and establish severe infections. Resistance to several antibiotic classes has been reported, making *K. pneumoniae* an increasing public health concern. We have recently identified 4 clinical isolates of *K. pneumoniae* sequence type (ST) 392 KPC3-producing strains from patients at the Istituto Mediterraneo per i Trapianti e Terapie ad Alta Specializzazione (IRCCS ISMETT), a Southern Italian transplantation health facility, during a routine surveillance for carbapenemase-producing *Enterobacteriaceae* from inhouse clinical samples.

Objectives: Since those were the first KPC-producing K. pneumoniae ST392 isolated in Europe, to the best of our knowledge, we assessed their virulence potential, to understand if this particular ST can become an endemic clinical threat.

Methods: ST392 strains were submitted to well-described in literature methods to assess their virulence potential, namely resistance to human sera, formation of abiotic and biotic biofilms and exopolysaccharide production.

Results: These strains were shown to be highly resistant to human sera and to have a high capacity to form abiotic biofilms but moderate adhesion to a human intestinal cell line. ST392 strains showed high levels of exopolysaccharide production, which relates with their biofilm formation aptitude. Interestingly, one ST392 isolate demonstrated to be hypermucousviscous, a characteristic shared by hypervirulent *K. pneumoniae* strains. We are currently studying the role of complement deposition and transcriptional levels of molecules involved in sera resistance to further understand the virulence potential of these recently identified ST392 *K. pneumoniae* strains.

PM251 Analysis of the microbiota from resistant and susceptible Rainbow trout (Oncorhynchus mykiss) and isolation of microorganisms with antimicrobial capacity against Flavobacterium psychrophilum

Mario Tello[1;2], Natalia Valdes[1;2], Claudia Zapata[1;2], Mick Parra[1;2], Alex Gonzalez[3]

[1]*Centro de Biotecnología Acuicola-USACH, Biologia, Santiago, Chile*
[2]*Facultad de Química y Biología, Biologia, Santiago, Chile*
[3]*Laboratorio de Microbiología Ambiental-Ulagos, Chile*

Background: The microbiota plays an important role in the protection of organisms against bacterial infections. Among the main pathogens affecting the salmon industry is *Flavobacterium psychrophilum*, the bacterium that causes the bacterial cold water disease (BCWD).

Objectives: The goal of this study is to determine if there are differences between the microbiota of fishes resistant and susceptible to infection of *F. psychrophilum* and isolate microorganisms with antimicrobial activity against *F. psychrophilum.*

Methods: For the development of this study, a comparison was made of the microbiota, present in resistant and susceptible fish to *F. psychrophilum* and cultured microorganisms belonging to the microbiota of fish resistant to infection were isolated.

Results: The results show that the fish's microbiota susceptible to infection have a more diverse microbiota (266 different OTUs) than the microbiota of resistant fish (134 different OTUs). The microbiota of resistant fish presents a greater abundance of the bacterial genus *Muribaculum, Pseudomonas, Alloprevotella, Lactococcus* and *Carnobacterium* than the susceptible fish. In addition a total of 232 bacteria was isolated. From these microorganisms, tests of antagonism against the pathogen were carried out. Of the total of 15 isolates, they have the capacity to inhibit the growth of *F. psychrophilum*, generating haloes between 9 and 18 mm. The 15 isolates correspond to bacteria belonging to the genera *Pseudomonas, Comamonas, Flavobacterium* and *Chryseobacterium.* Altogether our results suggest that isolates of the genus *Pseudomonas* can be used as native probiotics for the salmon farming industry, protecting against mortality caused by BCWD.

PM252 Chlamydia trachomatis is susceptible to newly designed furanone inhibitors of Na+-NQR

Elena Dibrov[1], Jordan Nelson[1], Nastassja Prybylski[2], Thane G. Maddaford[1], Craig Resch[1], Kim A. O'Hara[1], Pavel Dibrov [3], Grant N. Pierce[1]

[1]St. Boniface Hospital Albrechtsen Research Centre, Institute of Cardiovascular Sciences, Winnipeg, Manitoba, Canada
[2]Université Blaise Pascal, Clermont-Ferrand II, Pascal Institute, France
[3]University of Manitoba, Department of microbiology, Winnipeg, Manitoba, Canada

Background: Bacterial resistance to antibiotics is one of the greatest threats that mankind faces today. Modern antibiotics inhibit a limited repertoire of targets and target-specific resistance has emerged against every antibiotic class. Na^+-translocating NADH-ubiquinone oxidoreductase (Na^+-NQR) is a major respiratory Na^+ pump in Gram-negative pathogens so that narrowly-targeted anti-NQR drugs could potentially be effective against a surprisingly broad spectrum of pathogens.

Objectives: To design Na^+-NQR inhibitors non-toxic for animal cells and to examine whether the inhibition of Na^+-NQR could disrupt chlamydial infections.

Methods: Infection of HeLa and HEK-293 cells by *C. trachomatis* was used as a model to assess antichlamydial activity of a novel synthetic furanone inhibitor of Na^+-NQR (PEG-2S) *via* immunocytochemistry. pHrodo™ Green AM and CoroNa Green Indicators were used to measure intracellular pH and intracellular sodium concentration, respectively.

Results: Anti-Na^+-NQR compound PEG-2S inhibits the ability of C. *trachomatis* to perturb ionic homeostasis of infected cells. A number of furanone inhibitors of Na^+-NQR were designed and tested on the HELA cells infected with *C. trachomatis*. PEG-2S and PEG-6 were found to be most effective against *C. trachomatis* infection. Minimum Inhibitory Concentrations (MIC) and Minimal Chlamydicidal Concentrations (MCC) for PEG-2S were 0.7μM and 1.7μM respectively. MIC and MCC for PEG-6 were 0.56μM and 1.73μM. The Chlamydia Recovery Rate was established after two consecutive treatments with PEG-2S and PEG-6 at 1μM and 1.5μM. Our data confirm that Na^+-NQR is indispensable for the *Chlamydiae* infectious process and inhibitors of Na^+-NQR could be used as a platform for developing new antibiotics.

PM253 The Conformation of Adsorbed Fibronectin Determines the Success of Bacterial Attachment

Nasar Khan[1], Hüsnü Aslan[1], Henning Buettner[2], Holger Rohde[2], Rikke Louise Meyer[1]

[1]*Aarhus University, Interdisciplinary Nanoscience Center (INANO), Aarhus, Denmark*
[2]*University Medical Center Hamburg-Eppendorf, Medical Microbiology, Virology and Hygiene, Hamburg, Germany*

Background: *Staphylococcus epidermidis* is responsible for many implant-associated infections due to its ability to form biofilms. It attaches to implants by interacting with adsorbed host proteins. One prominent adhesin is the extracellular matrix binding protein (Embp), which binds to fibronectin (Fn).

Objectives: Host proteins like Fn are present in body fluids as well as on implant surfaces, so how can they promote attachment? We hypothesized that Embp only attaches to immobilized Fn because its binding domains only become available when Fn changes conformation upon adsorption to the implant. Soluble Fn is globular while adsorbed Fn undergoes conformational changes that sometimes lead to fibrillation.

Results: We first confirmed that Embp only binds to adsorbed Fn. To study the role of Fn conformation in this interaction, we produced a model system in which Fn adsorbed to polymer-coated surfaces in either globular or fibrillar conformation. This was achieved on (poly)methyl acrylate (PMA) and (poly)ethyl acrylate (PEA), respectively. Atomic force microscopy confirmed that Fn only fibrillated on PEA. We quantified Embp-mediated bacterial attachment to Fn on these two surfaces, using *Staphylococcus carnosus* expressing recombinant Embp. Fibrillar Fn promoted bacterial attachment while globular Fn did not. Furthermore, single cell force spectroscopy showed much stronger adhesion forces to surfaces with fibrillated Fn. Our study is the first to show that adsorption-induced conformational changes dictate if a host protein can serve as an anchor for bacterial attachment, and this insight adds a new layer to considerations made in materials design for implant materials that prevent biofilm infections.

PM254 Detection of mcr-1, blakPC and blaCTX producing Pseudomonas aeruginosa from food producing animals in Southwest Farms In Nigeria

Olayemi Oladapo[1]

[1]*University of Lagos, Microbiology, Lagos, Nigeria*

Background: Antibiotic resistance (AR) in bacteria is a current global crisis that needs urgent preventive methods by detecting its various sources. Food producing animals harbouring AR bacteria has been identified as a potential threat to the public health. In Nigeria, less attention is paid to the zoonotic source of transmission of AR bacteria to human via food-producing animals and their companions.

Objectives: To detect the presence of mobilized AR genes in resistant *Pseudomonas aeruginosa* isolated from food-producing animals comprising of cattle, pigs, goats, and chicken from different farms in Lagos and Ogun state Nigeria.

Methods: A total of 95 *P. aeruginosa* were isolated from 50 faecal samples each from the target animals. They were further investigated for susceptibility to antibiotics and the presence of AR genes and class 1 integrons by disk diffusion method and Polymerase Chain Reaction (PCR) respectively.

Results: Complete resistance (100%) was observed for cefotaxime and ceftazidime followed by 69.5% for meropenem. There was complete susceptibility to imipenem, cefepime, piperacillin/tazobactam, and amikacin while 94.7% were susceptible to ciprofloxacin, 88.4% colistin. Among the 22 randomly selected for PCR amplification, 4 (18%) was positive for bla_{KPC}, a single strain harboured both bla_{CTX} and class 1 integron while another strain harboured mcr-1. This is the first description of mcr-1 in *P. aeruginosa* in Nigeria. This study ascertains the carriage of multiple mechanisms of AR among of *P. aeruginosa*, hence predicts a high risk of transmission to humans through consumption of undercooked meat product.

PM255 Multiparametric profiling strategy for evaluating novel antivirulence compounds against uropathogenic E.coli

Polina Ilina[1], Aleksi Ainonen[1], Blanca Gonzalez Melarde[1], Cristina Durante Cruz[1], Päivi Tammela[1]

[1]*University of Helsinki, Faculty of Pharmacy, Helsinki, Finland*

Background: Uropathogenic Escherichia coli (UPEC) is the leading cause of urinary tract infections. Due to increasing antibiotic-resistance, alternative therapies are urgently needed. One promising approach is to design antivirulence compounds aimed to disarm bacterial pathogens. However, such compounds do not affect bacterial viability, and therefore cannot be discovered using conventional antibacterial assays.

Objectives: We aimed to set up a whole cell-based assay platform for multiparametric evaluation of antivirulence properties of small molecular compounds against UPEC infection. We furthermore characterized a set of compounds with known quorum sensing inhibitory activity for their potential use as reference compounds in studies aimed to discover novel quorum sensing inhibitors.

Methods: We set up a qPCR assay for a panel of over 15 genes known to be involved in UPEC pathogenicity in order to evaluate the effect of compounds at gene expression level. As biofilm formation is one of the key features in UPEC virulence, we optimized a range of biofilm quantification assays such as crystal violet, resazurin assay and bacterial colony counting. Uroepithelial cells were used to set up pathogen-to-host adherence and invasion (gentamicin protection) assay.

Results: The developed assay platform allows detection of antivirulence properties of potential drug candidates at gene expression and phenotypic level. Compounds with previously demonstrated quorum sensing inhibitory activity demonstrated only minor effect on UPEC virulence, possibly due to enhanced efflux pump activity in the wild type strains used. Therefore, we will continue searching for more efficient reference compounds.

PM256 The Impact of Staphylococcus aureus Phages as a Survival Strategy

Ahlam Alsaadi[1], Jakob Haaber [1], Hanne Ingmer [1]

[1]*University of Copenhagen, Health and Medical Science , Copenhagen, Denmark*

Background: Antibiotics resistance has emerged as a major global health threat, undermining our ability to treat infectious diseases. Resistant genes are transferred between bacterial strains with a high frequency by horizontal gene transfer. Bacteriophages are a class of mobile genetic elements that efficiently mediate the transfer of various genes in the human pathogens, *Staphylococcus aureus* , a common cause of infection in both hospitals and the community. *S.aureus* carry lysogenic phages that have the ability to occasionally package bacterial DNA within phage particles and subsequently transfer the DNA between strains, by a mechanism known as generalized transduction. Thus, phages are primary forces that drive the dissemination of virulence and antibiotic resistance genes within a population and as such contribute to the emergence of new strains within serious healthcare implications.

Objectives: Here, we have been interested in the transducing temperate phages that integrate into the bacterial chromosome and survive the phage killing by becoming lysogens.

Methods: We prepared different bacterial strains with various resistance markers, phages (lytic and lysogenic) then we tested generalized transduction. We also detected the exchanged genetic materials under chosen conditions.

Results: Our recent results suggest that temperate transducing phages enable their hosts to survive an exposure to antibiotics through concomitant transduction and lysogenization. Under antibiotics pressure, through transduction, temperate phages benefit from transducing particles to shuffle antibiotic resistance genes between strains. Therefore, we propose that transduction is an evolutionary trait that allows temperate phages surviving in rapidly-changing environment and lead to the dissemination of antibiotic resistance genes.

PM257 Analysis of the global aminoglycosides resistome of Pseudomonas aeruginosa allows defining genes simultaneously involved in intrinsic antibiotic resistance and virulence

Fernando Sanz[1], Carolina Álvarez[1], Jorge Olivares[1], Paula Blanco[1], Jose Luis Martinez[1], Sara Hernando-Amado[1]

[1]*Centro Nacional de Biotecnología, Biotecnología Microbiana, Madrid, Spain*

Background: Screening of transposon insertion libraries is a useful strategy for unveiling bacterial genes whose inactivation drives to an altered susceptibility to antibiotics and for the characterization of targets whose inhibition could simultaneously suppress the expression of virulence factors and antibiotic resistance. A potential drawback of these studies is the fact that they are usually based on the use of just one model antibiotic for each structural family, under the assumption that the results can be extrapolated to all members of such family.

Objectives:

- To determine if the above-mentioned simplification is appropriate in the case of aminoglycosides family and *P. aeruginosa*.
- To search for genes that could be simultaneously involved in antibiotic resistance and virulence in *P. aeruginosa.*

Methods: We analysed the susceptibility of a set of 243 mutants of *P. aeruginosa*, some previously described to be involved in aminoglycosides resistance, some coming from an *in-house* screening, to four aminoglycosides.

Five aminoglycosides hypersusceptible mutants inactivating *glnD, hflK, PA2798, PA3016* and *PA4463* were chosen for further susceptibility and virulence analysis.

Results: Our results indicate that each mutation produces different effects in the susceptibility to the tested aminoglycosides. This shows that the role of a particular gene in the resistome of a given antibiotic should not be generalized to other members of the same family. The inactivation of *glnD* leads to an increased cross-susceptibility to different antibiotics, and strongly impaired *P. aeruginosa* virulence. GlnD might be an interesting target for developing coadjunvants with anti-resistance and anti-virulence properties against *P. aeruginosa*.

PM258 Metagenomic sequencing analysis of stool sample of diarrhea patients in Kolkata: Indication of carrier of Vibrio cholerae

Keinosuke Okamoto[1], Eizo Takahashi[2], Shin-ichi Miyoshi[3], Asish Mukhopadhyay[4], Goutam Chowdhury[4], Tetsuya Iida [5]

[1]*Collaborative Research Center of Okayama University for Infectious Diseases in India, Kolkata, India*
[2]*Collaborative Research Center of Okayama University for Infectious Diseases in India, Okayama University, Kolkata, India*
[3]*Okayama University, Okayama, Japan*
[4]*National Institute of Cholera and Enteric Diseases, Kolkata, India*
[5]*Research Institute for Microbial Diseases, Osaka University, Suta, Japan*

Background: The diagnosis of infection of *Vibrio cholerae* O1 is determined by isolation of *V. cholerae* O1 from stool by bacteriological examination. However, it remains unclear whether all patients cholera have been detected by the examination.

Objectives: In order to examine whether the bacteriological examination detects accurately patients cholera, metagenomic analysis is provided to find out *V. cholerae* O1 in stools of diarrhea patients

Methods: Thirty six stool samples of diarrhea patients in Kolkata , India were analyzed by metagenomic analysis. In addition, the amount of cholera toxin (CT) in these stools was measured.

Results: By laboratory examination, *V. cholerae* O1 were isolated from 8 samples. However, metagenomic analysis of these 8 samples showed that the genes derived from *V. cholerae* O1 were not detected in 3 samples, indicating that these 3 patients were not infected with *V. cholerae* O1. Subsequently, we examined choler toxin (CT) in these stool samples. As well as in the metagenomic analysis, CT was not detected in these 3 samples. However, the isolates from these 3 samples produce CT outside of the cells *in vitro*. It suggests that toxigenic *V. cholerae* O1 inhabit the gut of these 3 persons, but do not act to induce diarrhea. This means these persons might be carriers of the bacteria. The metagenomic analysis of these remnants of 28 stool samples showed that 4 samples contain the genes from *V. cholerae* with significant high value. This supports the idea that carrier persons are there in Kolkata.

PM259 Frequent emergence of sigB mutant alleles in Listeria monocytogenes is likely to be linked to a growth advantage at elevated temperatures

Duarte Guerreiro[1], Charlotte Dessaux[2], Catarina Moreira Marinho[1;3;4], Teresa Tiensuu[5], Franscisco García-del Portillo[2], Jörgen Johansson[5], Conor O'Byrne[1]

[1]*National University of Ireland, Galway, Bacterial Stress Response Group, Galway, Ireland*
[2]*Centro Nacional de Biotecnología, Consejo Superior de Investigaciones Científicas (CSIC), Madrid, Spain*
[3]*Université de Bourgogne, Dijon, France*
[4]*Institut National de la Recherche Agronomique (INRA), UMR 1347 Agroécologie, Dijon,*
[5]*Umeå University, Department of Molecular Biology, Umeå, Sweden*

Background: The food-borne pathogen *Listeria monocytogenes* is a Gram-positive bacteria and highly resistant to a variety of stresses. Such resistance is determinate by the activation of the alternative sigma factor B (σ^B). Genes within *sigB* operon encode for the signal transduction that controls σ^B activity. Mutations within this operon generally result on reduced σ^B activation.

Objectives: Characterize the impact of secondary mutations within *L. monocytogenes sigB* operon, and identify laboratory conditions that promote the selection of such mutations among wild type (WT) populations.

Methods: Transposon mutants (Tms) that lost the ability to form rings in response to cycles of light and dark were analyzed through whole genome sequencing. Challenged for acid tolerance (pH 2.5). σ^B activity was measured using the EGFP reporter fusion system. Growth kinetics measured at different temperatures. Competition experiments were performed using mixed cultures incubated at 42°C.

Results: Frameshift mutations within the *sigB* operon were identified in Tms, which showed decreased acid tolerance and less fluorescence in the EGFP reporter strains, demonstrating a reduced σ^B activation. Increased growth rates of Tms and Δ*sigB* when compared to WT during the transition to stationary phase at elevated temperatures (40-42°C). Tms and Δ*sigB* were able to overtake the WT population overtime, but not vice versa, in competition experiments. Elevated growth temperature can result in the selection for mutations that result in deceased σ^B activity in *L. monocytogenes*. Researchers use temperature based selection strategies should be cognizant of this finding during routine culture and genetic work on this organism.

PM260 Differential Adhesion of Trimeric Autotransporter Adhesins to Extracellular Matrix Proteins

Ina Meuskens[1], Dirk Linke[1], Tim Gibson[2], Paul Millner[3]

[1]*University of Oslo, Department of Biosciences, Oslo, Norway*
[2]*ELISHA Systems Ltd, Stanley, United Kingdom*
[3]*University of Leeds, Faculty of Biological Sciences, Leeds, United Kingdom*

Background: Bacterial infections follow a concerted pathway of initial adhesion, tissues invasion and perseverance in the host. Trimeric autotransporters (TAAs) are outer membrane virulence factors conferring adhesive properties to different surfaces including extracellular matrix proteins (ECM). The substrate specificity is still poorly understood. The prototypical TAA YadA (*Yersinia* adhesin A) binds to different ECM molecules in a serotype- specific manner (Mühlenkamp et al. 2017).

Objectives: YadA of *Yersinia enterocolitica* serotype O:9 harbors an additional stretch in the head which preferentially binds Vitronectin (Vn) while heads without the stretch have low affinities to Vn but to collagen (Nummelin et al. 2004). Whether this stretch adopts a fold during target binding is the key question of our research. A deeper understanding of adhesion to the host will contribute to better treatment of bacterial infections.

Methods: We employ NMR to show whether unfolded loops in some YadA heads account as intrinsically disordered polypeptides adopting a fold upon target interaction. Furthermore, we employ protein-protein interaction tools like SPR and MST to get a clear picture of the exact. We additionally plan to map the interactions via phage display. Since we are part of a European innovative training network we collaborate with the Institute Pasteur for NMR studies and with the companies ELISHA and ELUCEDA to develop biosensors for diagnostics.

Results: Initial bindiging experiments with fluorescently labeled bacteria expressing full length constructs of YadA from different serotypes show promising differences in binding to a variety of ECM molecules. Biosensor expriments are planned in preperation.

PM261 Combination of Cannabidiol and Bacitracin Against Bacterial Infections

Claes S. Wassmann[1], Janne Kudsk Klitgaard[1]

[1]*University Of Southern Denmark, Biochemistry and Molecular Biology, Odense, Denmark*

Background: Development of antibiotic resistance has become a major problem world-wide making many conventional antibiotic useless. Therefore, we need to turn to other approaches such as use of helper compounds. Helper compounds usually possess poor antimicrobial effects themselves, but are able to potentiate antibiotics against resistant bacteria.

In our research group, we have discovered that the cannabinoid cannabidiol (CBD) is able to potentiate the effect of bacitracin against Methicillin-Resistant *Staphylococcus aureus* (MRSA) and other Gram-positive bacteria.

Objectives: In this project, we aim to discover the mechanism underlying the CBD mediated potentiation of bacitracin against MRSA and other Gram-positive bacteria and using this knowledge to develop a treatment for skin infections.

Methods: Growth experiments have shown that CBD is able to potentiate the effect of bacitracin against MRSA and other Gram-positive bacteria such as *Listeria monocytogenes* and *Enterococcus faecalis*. CBD has shown to cause disruption of the membrane potential of MRSA and when combined with bacitracin, transmission electron microscopy pictures revealed an effect on the membrane visualized by multiple septum formation and membrane irregularities which may affect the divisome. Furthermore, RNA-seq data have shown several transporters and efflux pumps to be differential regulated upon treatment.

Results: Our data show that CBD can potentiate the effect of bacitracin against Gram-positive bacteria. The mechanism underlying the potentiation is still unknown, but our results points towards the membrane to be an important area, however, the exact mechanism will be of focus for our future research.

PM262 Prevalence and determination of significant titre values for diagnosis of typhoid fever in Enugu State, Nigeria

Nkechi Chiaha[1]

[1]*University of Nigeria, Department of Biochemistry, Nsukka, Nigeria*

Background: Typhoid fever is endemic in Nigeria. It is a major public health problem in developing countries due to poor sanitary conditions or lack of potable water. Cross reactivity with other bacterial and protozoan infections like malaria, makes the Widal test, a serodiagnostic tool for this fever, doubtful.

Objectives: The objectives are to evaluate the prevalence of typhoid fever among blood donors and to determine the significant titer value for diagnosis of enteric fever by the use of analysis of result from normal population.

Methods: The sera from blood donors were subjected to Widal agglutination tests using commercial available antigens. Serum samples from 250 blood donors were tested for IgG/1gM antilipopolysaccharide antibodies of *Salmonella* by ELISA.

Results: Out Of the 250 blood samples 148 (59.2%) were positive following a Widal agglutination test, 53 (21.2%) blood samples grew *Salmonella* organisms on blood culture while 49 (19.6%) blood samples showed a co-infection of typhoid fever and malaria. The severity of malaria parasitemia is associated with positive titers on Widal test. Therefore, a single Widal agglutination test is not a valid diagnostic option for typhoid fever while co-infection with malaria parasite is the preponderant microbiological finding in typhoid fever infections. The majority of the normal healthy individuals also carry detectable antibodies due to the repeated prior exposure with low inocula of typhoid bacilli. Therefore, the reliability of the result of single serum sample serological test should be based on a reference baseline and cut-off value of healthy population in a geographical area.

PM263 The lipopolysaccharide of Burkholderia glumae plays a role as virulence factor and a barrier from external environment

Namgyu Kim[1], Chaeyeong Lee[1], Gil Han[1], Hyun-Hee Lee[1], Inmyoung Park[2], Young-Su Seo[1]

[1]Busan, Department of Integrated Biological Science, Pusan National University, Busan, Korea, Rep. of South
[2]Busan, Department of Oriental Food and Culinary Arts, Youngsan University, Busan, Korea, Rep. of South

Background: *Burkholderia glumae* BGR1 is a Gram-negative plant pathogen and has been shown to cause panicle blight to rice. Gram-negative bacteria have lipopolysaccharide (LPS) composed of the three parts: lipid A, core-oligosaccharide (core-OS), and O-antigen. Core-OS attached between lipid A and O-antigen is divided into an inner-core and an outer-core, which is structurally distinct from other Gram-negative bacteria.

Objectives: LPS is known to be a barrier from the outside environment because it is located in the bacterial outer membrane, and therefore the susceptibility changes due to LPS deficiency under antimicrobial peptide materials or various external stress conditions. The role of LPS in *B. glumae* was investigated by mutating *wbiFGHI* operon and *waaC* that are affected to O-antigen and core-OS synthesis, respectively.

Methods: Electrophoretic analysis of LPS, *in vitro* phenotype assay

Results: A core-OS mutant was more sensitive against antimicrobial peptide substances and under various environmental stress conditions. Each mutation of *wbiFGHI* operon gene showed a similar response like wild-type under all conditions tested. In terms of virulence and motility, O-antigen mutants did not differ greatly in motility and disease severity on rice, but the motility and virulence of the core-OS mutant strain was greatly reduced. This decrease in virulence may be due to reduced motility or the antimicrobial peptides of the host on the weakened outer membrane. Based on these results, LPS of *B. glumae* can act as a barrier in the bacterial outer membrane against antimicrobial peptides and various stress conditions as well as a virulence factor.

PM264 Characterization of a Chlamydia trachomatis protein that targets host cell lipid droplets

Joana Bugalhão[1], Maria Luís[1], Adriana Vieira[1], Luís Jaime Mota[1]

[1]*Faculty of Sciences and Technology, NOVA University of Lisbon, UCIBIO, Department of Life Sciences, Caparica, Portugal*

Background: *Chlamydia trachomatis* is the leading cause of sexually transmitted bacterial diseases. In women, it can cause infertility and ectopic pregnancy. This obligate intracellular human pathogen thrives within a membrane-bound compartment, the inclusion. *Chlamydia* manipulates host cell trafficking by using bacterial effector proteins delivered into the host cell by a type III secretion system. These effectors include inclusion membrane (Inc) proteins with important roles in *Chlamydia*-host cell interactions.

Objectives: We aimed to identify and characterize *C. trachomatis* Inc proteins subverting host vesicular trafficking and/or showing tropism for eukaryotic organelles.

Methods: We performed a functional screen and fluorescence microscopy analysis using the yeast *Saccharomyces cerevisiae* ectopically expressing Inc proteins. Based on these screens we selected one Inc for a detailed characterization in mammalian cells using molecular biology and biochemical techniques.

Results: We identified two Incs causing vacuolar protein sorting defects in yeast. Furthermore, we found one Inc showing tropism for lipid droplets (LDs), major organelles for the storage of neutral lipids and which have been shown to be recruited to the *Chlamydia* inclusion during infection. We hypothesize that this Inc might have an important role in this process. Using mammalian cells ectopically expressing truncated versions of the Inc protein, we identified a region responsible for targeting the protein to LDs. In addition, co-immunoprecipitation assays showed that this Inc bound 14-3-3β after ectopic expression in mammalian cells. We are currently generating a *C. trachomatis* mutant for the gene encoding this Inc, in order to understand its biological role during infection.

PM265 Identification and characterization of novel type 4 secretion effectors in a Legionella pneumophila strain recently isolated from a major outbreak in Portugal

Inês Pais[1], Vitor Borges[2], Paulo Gonçalves[2], João Paulo Gomes[2], Jaime Mota[1], Irina Franco[1]

[1]UCIBIO-REQUIMTE, Faculdade de Ciências e Tecnologia, Universidade NOVA de Lisboa, Department of Life Sciences, Caparica, Portugal
[2]Instituto Nacional de Saúde Doutor Ricardo Jorge, Department of Infectious Diseases, Lisboa, Portugal

Background: *Legionella pneumophila* is a Gram-negative bacterium that may infect human lung macrophages causing a severe type of pneumonia known as Legionnaires' disease. The second largest Legionnaires' disease outbreak worldwide originated the first case of probable person-to-person transmission and was caused by a strain with a new genetic backbone, containing numerous strain-specific genomic islands associated with virulence.

The major virulence mechanism of *L. pneumophila* is the Icm/Dot type 4 secretion system (T4SS), that translocates into host cells over 300 substrates. Identification of these effectors is crucial but has been hindered by the absence of infection-related phenotypes resulting from effector gene knockout. Thus, new insights on both conserved and strain-specific virulence traits may arise from analysing effector functions in new genomes.

Objectives: We aimed to identify and functionally characterize new T4SS effectors encoded in the genome of the novel *L. pneumophila* strain.

Methods: To identify new effector proteins we carried out a bioinformatics search and the obtained hits were validated using a FRET-based assay. Bona fide effectors were analysed for their subcellular localization in transfected mammalian CHO cells as fusions to the C-terminus of EGFP.

Results: Using the FRET-based translocation assay, we identified two new substrates of the *L. pneumophila* Icm/Dot T4SS. When expressed in CHO cells, one of these effectors was homogeneously distributed in the cytosol, whereas the other was targeted to the nucleus. For the latter, we have identified a C-terminal region comprising ~50 amino acid residues as necessary for its nuclear localization.

PM266 Staphylococcus aureus Phenol Soluble Modulins: Role in functional amyloid, biofilm and persister cell formation

Mara Baldry[1], Brian Stougaard Vad[2], Daniel Otzen[2], Rikke Louise Meyer[2], Hanne Ingmer[1]

[1]University of Copenhagen, Department of Veterinary and Animal Sciences, Faculty of Health and Medical Science, Frederiksberg C, Denmark
[2]Aarhus University, iNANO, Aarhus C, Denmark

Background: Amyloid fibrils are well known for their role in degenerative diseases in mammals (e.g. Alzheimer's), however, their presence has also been reported in microorganisms where they serve a functional role often associated with biofilms. In *Staphylococcus aureus* the Phenol Soluble Modulins have been described as capable of forming amyloid fibrils, and this property is suggested to increase *S. aureus* biofilm integrity. While data exists to support fibril formation of synthetic PSM peptides *in vitro*, there is considerable lack of evidence that this phenomenon is truly integral to a biofilm setting.

Objectives: The objectives of this study were to set-up methods for detecting staphylococcal amyloids produced during biofilm, identify new amyloid proteins in *S. aureus*, and define the role of PSM amyloids in staphylococcal biofilms.

Methods: *S. aureus* and isogenic PSM mutants were grown under flow or static conditions and amyloid detection in the resulting biofilms was attempted by staining with amyloid-specific dyes, microscopy, and antibody recognition. The effect of amyloid inhibitors (e.g. EGCG, PGG) and matrix degrading enzymes were also studied to further describe biofilm integrity related to PSMs. Lastly the role of synthetic PSM fibrils in persister cell formation in biofilms was also investigated.

Results: Significant challenges were met when trying to detect *S. aureus* PSM amyloid fibrils in biofilm material. While we conclude that PSMs are not likely an integral amyloid component of *S. aureus* biofilm, our data does suggest that there are some amyloid-like components present in *S. aureus* biofilm that still remain to be characterised.

PM267 Impact of mesophilic anaerobic digestion on toxinotype of Clostridium perfringens strains and their antibiotic resistance

Lorine Derongs[1], Céline Druilhe[1], Laure Martin[2], Caroline Le maréchal[2], Martine Denis[2], Anne-Marie Pourcher[1]

[1]*irstea, OPAALE unit, Rennes, France*
[2]*anses, HQPAP unit, Ploufragan, France*

Background: The development of mesophilic anaerobic digestion leads to a high amount of digestate which may contain pathogens. Among them, *Clostridium perfringens*, which produces several toxins, deserves special attention due to its ability to multiply under mesophilic anaerobic conditions.

Objectives: The study aims to (i) enumerate *C. perfringens* and (ii) characterize the toxinotype and evaluate the antimicrobial resistance of strains of *C. perfringens* isolated from manures and raw digestates.

Methods: *C. perfringens* was quantified by cultural method over a one-year period in manure and raw digestate from 3 biogas plants. Two of them treated swine manure, the third one treated cattle manure (85% of the manure input) and poultry (15%). A total of 170 presumptive strains were characterized by searching the most common major and minor toxin genes of *C. perfringens*. The antimicrobial susceptibility was determined on 18 antibiotics commonly used in livestock.

Results: This study is the first to investigate the fate of *C. perfringens* toxinotypes and their resistance to antibiotics in manures and raw digestates. Mesophilic anaerobic digestion did not impact *C. perfringens* concentration. Toxinotype A which is responsible for gas gangrene or intestinal disease in human and animal, is predominant in both manure and raw digestate. The resistance profiles of the strains did not differ according to their origin (manure vs digestate). However, strains originated from cattle and swine manure were more resistant to the 18 antibiotics than those isolated from the poultry manure. Moreover, multi-resistant *C. perfringens* are present in raw digestate.

PM268 Genotypic and phenotypic characterization of an uropathogenic Escherichia coli strain presenting virulence properties of enteroaggregative E. coli

Paulo Schüroff[1], Eneas de Carvalho[1], Cecilia Mari Abe[1], Tania Aparecida Tardelli Gomes[2], Waldir Elias[1]

[1]*Instituto Butantan, Laboratório de Bacteriologia, São Paulo, Brazil*
[2]*Universidade Federal de São Paulo, Disciplina de Microbiologia, São Paulo, Brazil*

Background: Uropathogenic *Escherichia col*i (UPEC) is the most important agent of urinary tract infection (UTI). Recently, enteroaggregative *E. coli* (EAEC) strains have been detected as causative agents of UTI. Previously, we detected a *E. coli* strain isolated from UTI (UPEC-46) presenting characteristics of EAEC, *e.g.* the aggregative adherence (AA) pattern in HeLa cells and the presence of EAEC-associated genes (*aat*, *aap*, *astA* and *pet*).

Objectives: To analyze the whole genomic sequence of UPEC-46 and characterize some phenotypic traits.

Methods: DNA sequence was analyzed using SerotypeFinder, ResFinder and ecoli VF collection databases. Biofilm formation was evaluated in time course assays, using bacteria grown in Dulbecco's modified Eagle medium (DMEM) or preconditioned DMEM, on glass or polystyrene surfaces. Expression of Curli fimbria and Pet toxin, and adherence to HEK-293 cells were assayed.

Results: UPEC-46 belongs to the O166:H12 serotype and harbors *tet*, *aadA* and *dfrA*/*sul* resistance genes. A total of 129 *E. coli* virulence associated genes were found: 83 associated with adhesion and invasion, 30 with iron uptake and 8 with serum resistance. Also, 6 genes encoding autotransporter proteins, including *pet*, and 2 encoding toxins (*astA* and *hlyE*) were found. Production of Curli and Pet were detected, and AA was observed in HEK-293 cells. Furthermore, UPEC-46 was unable to form biofilm in all conditions tested. This study provides a more detailed understanding of the genetic background and phenotypic traits of an unusual *E. coli* strain and may contribute to elucidate which genetic elements are enabling an UTI pathogen to display EAEC properties.

PM269 The role of mannose-resistant type 1 fimbriae (MR T1Fs) in the invasiveness, survival and cytotoxicity of Salmonella Gallinarum in chicken macrophage-like HD11 cells

Agata Mikolajczyk[1], Aleksandra Tabis[2], Rafal Kolenda[1], Veria Khosrawipour[3], Maciej Ugorski[1]

[1]*Wrocław University of Environmental and Life Sciences, Biochemistry and Molecular Biology, Wrocław, Poland*
[2]*Wrocław University of Environmental and Life Sciences, Department of Food Hygiene and Consumer Health Protection, Wrocław, Poland*
[3]*Ortho-Klinik Dortmund, Department of Orthopedic and Trauma Surgery, Dortmund, Germany*

Background: *S.* Gallinarum, in contrast to most *Salmonella* serovars, express T1Fs that do not bind to oligomannosidic structures and therefore are called mannose-resistant T1Fs (MR T1F). However, it was shown that MR T1Fs mediate adherence of *S*. Gallinarum to chicken leukocytes and contribute to its virulence during systemic infection of 1-day-old chicks. This suggests that MR T1Fs may play an important role in the pathogenesis of *S*.Gallinarum infection. Macrophages transport bacteria from the intestine to the liver and spleen. Therefore, it is believed that the ability of *S*. Gallinarum to invade, multiply, and survive within these cells is necessary to elicit the systemic phase of infection.

Objectives: The aim of this study was to investigate the role of *S.*Gallinarum MR T1F in the invasiveness, survival and cytotoxicity in chicken monocytes and macrophages.

Methods: HD11 cells (non-stimulated or activated by lipopolisacharyde or phorbol mirystate-acetate to transform into macrophages) were infected with *S*. Gallinarum isolate nr 589/02 expressing MR T1Fs and S.Gallinarum Δ*fimH*::*kan* mutant non-expressing MR T1F. The invasiveness and survival of bacteria inside HD11 cells was analyzed using gentamycin protection assay. Lactate dehydrogenase (LDH) assay was used to assess the cytotoxic effects of bacteria toward cells.

Results: S.Gallinarum *fimH*::*kan* mutant non-expressing MR T1Fs was about ten times more invasive than wild-type *S*. Gallinarum expressing MR T1Fs, regardless of the functional state of the cells. However, the survival of bacteria inside host cells and their cytotoxicity did not depend on the presence of MRT1F.

PM270 The role of gene lmo0946 of Listeria monocytogenes in pathogenesis and environmental stress response

Emilia Prochwicz[1], Agata Krawczyk-Balska[1]

[1]*University of Warsaw, Department of Applied Microbiology, Faculty of Biology, Biological and Chemical Research Centre, Warsaw, Poland*

Background: *Listeria monocytogenes* being a foodborne opportunistic pathogen causes series of diseases from febrile listerial gastroenteritis to meningitides all described under the name – listeriosis. Recently have been described new gene encoding protein ferritin (Fri), involved in antibiotic resistance, environmental stress response and pathogenesis process. Its genetic organisation showed an operon structure with two upstream genes *lmo0944* and *lmo0945.* Further researches showed that also another gene *lmo0946* is also cotranscribed with *fri* gene. What is more function of protein Lmo0946 encoded in this *locus* is yet unknown.

Objectives: We aimed to investigate the role of *lmo0946* role in pathogenesis process and response to environmental stress.

Methods: The mutant in *lmo0946* gene was obtained and its capacity to sufficiently perform pathogenesis process were measured by its ability to survive inside mice macrophages. Also growth of *lmo0946* mutant under different environmental stress conditions was examined.

Results: Our research indicates that mutation of *lmo0946* gene impairs growth of *Listeria monocytogenes* under different stress conditions e.g oxidative and osmotic stress, acidic and alkaline environment. Furthermore this mutant shows significantly decreased survivability inside mice macrophages in comparison to *wild type* strain. This results point out that protein Lmo0946 plays important role in maintaining the fitness of *Listeria monocytogenes* both during saprophytic growth and infection process although the molecular basis of this protein activity remain unknown.

PM271 A distinct cysteine protease domain in the Vibrio vulnificus MARTX toxin

Sanghyeon Choi[1], Jungwon Hwang[1], Myung Hee Kim[1]

[1]Korea Research Institute of Bioscience and Biotechnology, Metabolic Regulation Research Center, Daejeon, Korea, Rep. of South

Background: Gram-negative bacterial pathogens deliver diverse effector proteins into the host cell via different secretion systems. Multifunctional autoprocessing repeats-in-toxin (MARTX) toxin is characterized as one of the delivery platforms for a repertoire of effector domains. Once secreted from bacteria via the atypical type 1 secretion system, MARTX toxins translocate into host cells and undergo an event to release the functionally discrete effector domains in the cytosol.

Objectives: It has been known that the internal cysteine protease domain (CPD) present in all MARTX toxins directs proteolytic processing of effector module following its activation and autoprocessing via binding with cellular inositol hexakisphosphate ($InsP_6$) that consequently exerts virulence by modifying cellular substrates. In this study, we identified a clinical strain of *Vibrio vulnificus* that possesses a CPD with unique amino acid sequences.

Methods: CPD proteins expressed in *E. coli* were purified using immobilized metal affinity chromatography (IMAC) and size-exclusion chromatography. The purified proteins were crystallized using sitting-drop vapor diffusion method. X-ray diffraction data of the CPD crystals were collected at beamline 5C of the Pohang Acceleratory Laboratory (PAL), Korea. The structure of CPD was determined by molecular replacement (MR) phasing and structure refinement using Phenix and Coot softwares.

Results: We determined the crystal structure of the CPD at a resolution of 2.2 Å. Structural analysis together with biochemical assays revealed that this CPD has distinct functions in the processing of MARTX toxin.

PM272 Adaptation to oxidative stress in pathogenic Leptospira: two is better than one!

Crispin Zavala-Alvarado[1], Nadia Benaroudj[1], Mathieu Picardeau[1], Jean-Yves Coppée[2], Hugo Varet[2], Odile Sismeiro[2]

[1]*Institut Pasteur, Microbiology, Paris, France*
[2]*Institut Pasteur, Genomes and Genetics, Paris, France*

Background: The emerging infectious disease leptospirosis has a worldwide distribution, affecting mostly people from developing countries. More than one million cases of severe leptospirosis are currently reported annually in the world, with 10% mortality. Pathogenic leptospires colonize kidneys of reservoir hosts (mainly rodents) and through urine they are excreted into the environment. Human are then infected by contact with soil and water contaminated with the urine of these animals. The symptoms of this infection are flu-like to more severe cases with multiple organ failure and hemorrhages. Defenses against ROS are an important strategy evolved by pathogenic bacteria such as *Leptospira*. Peroxidase activities that catalyze peroxide breakdown are crucial for *Leptospira* virulence. In *Leptospira*, genes encoding defenses against ROS are under the control of the Peroxide stress Regulator, a metalloprotein from the Ferric uptake regulator family. We have previously characterized the structural and functional properties of PerR1 and by examining the genome of *L. interrogans*, we have identified a second putative PerR.

Objectives:

Delineate the function of PerR2

Explore its interplay with PerR1 in the *Leptospira* oxidative stress response and virulence.

Results: Peroxidases and heme-biosynthesis machineries are up-regulated when *Leptospira* cells are exposed to peroxide.

Comparing the peroxide stimulon with the PerR1 and PerR2 regulons indicates that not all members of the stimulon are under the control of the PerR regulators.

PerR1 and PerR2 regulatory networks do not overlap.

PerR1 and PerR2 repress defenses against peroxide and superoxide anion, respectively.

The inactivation of the two PerRs impaired *Leptospira* virulence.

PM273 The polymicrobial nature of irish milk from cows with mastitis as revealed by culture and sequencing

Angeliki Angelopoulou[1;2], ross holohan[2], mary c. rea[2;3], alicja k. warda[2], colin hill[1;2], r. paul ross[1;2;3]

[1]*University College Cork, School of Microbiology, Cork, Ireland*
[2]*APC Microbiome Ireland, University College Cork, Cork, Ireland*
[3]*Teagasc Food Research Centre, Moorepark, Fermoy, Ireland*

Background: Mastitis is the most persistent disease of dairy cows and is defined as an inflammation of the udder. It causes economic losses due to depleted milk production, discarded milk, premature culling and treatment costs. Staphylococci, streptococci and coliforms are all among the most often identified microbial pathogens associated with the disease.

Objectives: This study aims at defining the microbiome composition of fifty mastitic bovine milks, employing both culture-dependent and culture-independent methods, and identifying the major pathogenic species associated with this infection.

Methods: Somatic cell counts were estimated by infrared analysis. Bacterial load was determined and causative pathogens were identified using culture-dependent techniques, Sanger sequencing and MiSeq sequencing.

Conclusions: The majority of samples were haemolysin positive and had over 10,000 cfu ml^{-1} total bacterial counts. Among colonies isolated on blood agar plates, streptococci were dominant (31.6%) followed by staphylococci (18.3%), *E. coli* (9.7%), enterococci (8.9%) and *Pseudomonas* sp. (8.2%) whereas colonies isolated from Baird Parker plates were identified as *S. argenteus* (19.5%), *S. aureus* (19.5%), *S. chromogenes,* (11.8%), *S. epidermidis* (8.3%) and *S. haemolyticus* (7.4%). Compositional sequencing revealed that bovine mastitic samples were dominated by *Rhodococcus, Staphylococcus, Streptococcus* and *Pseudomonas*. A high inter-sample diversity was noted in compositional sequencing results, which wasn't always reflected in the plating results.

These results suggest that a combination of bacterial culture methods in conjunction with compositional sequencing is required to get a true picture of this polymicrobial infection.

PM274 Identification and characterization of CteG, a new Chlamydia trachomatis effector protein

Sara Vilela Pais[1], Inês Serrano Pereira[1], Charlotte Key[2], Vítor Borges[3], João Paulo Gomes[3], Derek Fisher[2], Luís Jaime Mota[1]

[1]UCIBIO, NOVA University, Department of Life Sciences, Caparica, Portugal
[2]Southern Illinois University, Department of Microbiology, Carbondale, Illinois, United States
[3]National Institute of Health, Bioinformatics Unit, Department of Infectious Diseases, Lisbon, Portugal

Background: *Chlamydia trachomatis* is an obligate intracellular human pathogen causing ocular or genital infections. During its life cycle, *C. trachomatis* uses a type III secretion (T3S) system to deliver effector proteins into the host cell cytosol. These effectors enable bacterial survival and proliferation.

Objectives: Among previously identified *C. trachomatis* putative T3S effectors, we aimed to identify and characterize novel *C. trachomatis* effectors.

Methods: *C. trachomatis* strains expressing different double hemagglutinin (2HA)-tagged candidate effectors were used to identify effectors delivered into the cytoplasm of infected cells. We identified CteG as the first *C. trachomatis* effector associated with the Golgi and further studied its biological role using molecular and cellular biology techniques.

Results: CteG-2HA was delivered into the cytoplasm of infected cells by *C. trachomatis*. Whereas between 16-20 h post-infection CteG-2HA mostly associated with the Golgi, at 30 and 40 h post-infection it predominantly localized at the host cell plasma membrane. This change in the main localization of CteG-2HA was independent of intact microfilaments or microtubules. Ectopic expression of different regions of CteG in uninfected cells revealed that its first 100 residues contain a Golgi targeting region, and that its first 20 residues are necessary but not sufficient for this targeting. Although a *C. trachomatis cteG* mutant did not display a defect in intracellular multiplication, CteG induced a vacuolar protein sorting defect when expressed in *Saccharomyces cerevisiae*. This suggested that CteG might subvert host cell vesicular transport.

PM275 Role of Ly6G+ cells during Salmonella enterica serovar Typhimurium infection

Bárbara Schultz[1], Fernanda Balmaceda[1], Valentina Sebastián[1], Susan Bueno[1]

[1]*Millenium Institute on Immunology and Immunoteraphy. Pontificia Universidad Católica de Chile, Genética Molecular y Microbiología, Santiago, Chile*

Background: *Salmonella enterica* serovar Typhimurium (*S*. Typhimurium) is Gram-negative bacterium and an important gastrointestinal pathogen. To infect the host, *S.* Typhimurium has several virulence factors encoded in chromosomal cluster known as *Salmonella* Pathogenicity Islands (SPI). The most important are SPI-1 and SPI-2, which allow the infection of intestinal epithelial cells and the survival inside phagocytic cells, respectively. The initial innate immune response is the migration of neutrophils to the site of infection playing an important role by restricting the bacterial growth and dissemination. These cells attack the bacteria using different mechanisms, however, these are not enough to avoid *S*. Typhimurium dissemination. For this reason, it is possible that *S*. Typhimurium evade the function of neutrophils.

Objectives: To evaluate the neutrophil response against *S.* Typhimurium.

Methods: We isolate bone marrow derived neutrophils from male and female mice and evaluate the infection, bacterial survival, ROS and cytokine production and NETs release against *S.* Typhimurium *wild type*, Δ*spi-1* and Δ*spi-2* infection in order to evaluate the neutrophil response and evaluate if the virulence factor encoded in this SPI are relevant during the infection of these cells.

Results: *S.* Typhimurium is able to infect neutrophils and survive inside these cells up to 24 h, despite the intracellular ROS production and NETs induction by the bacteria. Importantly, the infected neutrophils begin to secrete the anti-inflammatory cytokine IL-10 at 24 hours post infection. Conclusion: The immune response of Ly6G+ against *S.* Typhimurium infection depends on the SPI that possess and the sex of the mice.

PM276 Acinetobacter baumannii multidrug-resistant: clonal determination using different technique and evaluation of MLVA to recognize international clone lineage,

Ilaria Unali[1], Claudia Thoma[1], Anna Bertoncelli[1], Annarita Mazzariol[1]

[1]*University of Verona, Department of Diagnostics and Public Health, Microbiology Section, Verona, Italy*

Background: *A. baumannii* is an important multi-drug-resistant opportunistic pathogen, and a leading cause of hospital-acquired infections associated with elevated morbidity and mortality.
Multi-drug resistance in A. baumannii is related to carbapenemases and aminoglycoside-modifying enzymes (AMEs) production.
A. baumannii infections are often associated with epidemic spread and its clonal population structure dominated by three international clonal lineages I–III.

Objectives: To determine the clonal relationship of *A. baumannii* strains isolated at the University Hospital of Verona and their international clone lineage. compared to other collections, trough three different techniques: PFGE, MLST, MLVA in order to check the last as easy, quickly and low-cost technique for further studies.

Methods: Seventeen carbapenems resistant *A. baumanni* strains were studied.
Antimicrobial susceptibility test was performed for amikacin, gentamicin, tobramycin; imipenem, meropenem and colistin.
All the isolates were characterized by the classic PCR and PCR-Multiplex techniques to search for genes that code for the major carbapenemases and AMEs produced by *A. baumannii*.
MLVA, PFGE and MLST were the techniques used to determine the clonal relatedness between strains.

Results: Strains carried $bla_{\text{OXA-23}}$, (58,8%). $bla_{\text{OXA-24}}$ and $bla_{\text{OXA-58,}}$ bla_{VIM} genes.
Most aminoglycoside resistant strains possess the *armA* gene (70,6%).
MLVA have highlighted 4 different molecular profiles. The 94,1% of the MLVA profiles were consistent with the other two methods used as comparison.
Strains isolated from Verona hospital belong to the international clone II since the two ST, namely ST1720 and ST218, belong to the CC92b. Our isolates confirmed the presence in the ICII of bla_{OXA23} and *armA*.

PM277 Extracellular low-molecular-weight phosphonates of phytopathogenic bacterium Pectobacterium atrosepticum

Olga Parfirova[1;2], Vladimir Gorshkov[1;2], Nadezhda Tarasova[1], Natalia Gogoleva[1;2], Andrey Smolobochkin[3], Olga Petrova[1], Yuri Gogolev[1;2]

[1]Kazan Institute of Biochemistry and Biophysics, Federal Research Center "Kazan Scientific Center of RAS", Kazan, Russian Federation
[2]Kazan (Volga region) Federal University, Kazan, Russian Federation
[3]A.E. Arbuzov Institute of Organic and Physical Chemistry, Federal Research Center "Kazan Scientific Center of RAS", Kazan, Russian Federation

Background: Phosphonates belong to an extensive group of compounds containing a carbon-phosphorus bond in their structure. Phosphonates can be included in macromolecules or represent low-molecular-weight compounds for which antibacterial and herbicidal properties are described. Plant soft rot-causing phytopathogen *Pectobacterium atrosepticum (Pba)* possess genes encoding the enzymes for biosynthesis and degradation of phosphonates. By RNA-Seq analysis we have shown that *Pba* phosphonate-related genes are up-regulated during soft-rot development compared to latent infection or cultures in vitro. However, Pectobacterium phosphonates are not described in literature.

Objectives: The aim of the present study was to obtain information on low-molecular-weight phosphonates and phosphonate-related genes in *Pba*.

Methods: Locus-specific mutagenesis was applied to obtain mutant for the phosphonate biosynthesis-related genes. NMR-spectroscopy was used for the detection of phosphanates in the supernatants of *Pba* cultures. Gene expression studies were carried out by qPCR and NGS.

Results: Mutant for phosphoenolpyruvate phosphomutase fom1 was obtained using three parental mating; the virulence of this mutant is currently characterized. To check if *Pba* indeed produces extacellular low-molecular-weigh phosphonates, *in vitro* conditions that induce the expression of phosphonate-related genes were selected. After culturing under these conditions, the supernatants were separated and the expected low-molecular-weight phosphonates were extracted. ^{31}P NMR spectra for the obtained samples revealed the 21.91 ppm signal typical of phosphonates as well as 59.69 ppm signal characteristic of phosphonium salts. Thus, we identified for the first time the low-molecular-weight phosphonates in *Pectobacterium* genus and obtained information on their role in pathogenesis. This study was supported by RSF (19-14-00194).

PM278 Analysing the Fibronectin Binding Domain of the Bartonella henselae Adhesin A

Arno Thibau[1], Diana Vaca[1], Dirk Linke[2], Volkhard A. J. Kempf[1]

[1]*Goethe University Hospital Fankfurt, Institute of Medical Microbiology, Frankfurt, Germany*
[2]*University of Oslo, Department of Biosciences, Oslo, Norway*

Background: *Bartonella henselae* infections cause cat scratch disease, endocarditis and vasculoproliferative diseases (e.g. bacillary angiomatosis). *Bartonella* adhesin A (BadA) mediates bacterial adhesion to human endothelial host cells (EC) or extracellular matrix (ECM) proteins (e.g. fibronectin). Fibronectin (Fn) is a high molecular weight glycoprotein that might mediate the initial bacterial adherence via bridging to β-1-integrins.

Objectives: The objective is to identify and modify the possible binding site(s) of BadA to Fn. Thereafter, peptides will be modelled and produced that inhibit bacterial adhesion to EC and ECM proteins. Additionally, a structural analysis of the BadA-protein will be performed. Read out will be done using various *ex vivo* and *in vitro* infection models. Findings might be used for developing bacterial "anti-ligands" as a new class of antibiotics.

Methods: Identification of the possible binding site(s) is performed via cloning-mutagenesis aiming for the production of truncated BadA fusion proteins (e.g. via Gibson cloning and/or via alanine scanning of the identified Fn-binding fragment). Structural analysis will be performed via cryo-electron microscopy.

Results: Previous research generated truncated BadA-fragments in which one specific fragment (F12) seems essential to bind Fn. Further analysis of this F12-fragment is ongoing. Generation of stalk-directed antibodies and a SacB-based mutagenesis strategy has been established.

PM279 Antibiotic resistance gene profile of fish pathogen Edwardsiella piscicida

Noemí Buján[1], Alicia E. Toranzo[1], Beatriz Magariños[1]

[1]Fac. Bioloxía-CIBUS e Instituto de Acuicultura, Universidade de Santiago de Compostela, Microbioloxía e Parasitoloxía, Santiago de Compostela, Spain

Background: *Edwardsiella piscicida* is an economically important fish pathogen especially in marine aquaculture over the world. At present, more than 20 antibiotics have been approved in Europe for fish farms, promoting the natural increase in antibiotic resistance. The importance of this bacterium in aquaculture made necessary to improve the knowledge of the genetic basis of the resistance to different compounds in order to stablish an effective control of edwardsiellosis disease.

Objectives: To investigate the antibiotic susceptibility and resistance gene profiles of the fish pathogen *E. piscicida*.

Methods: A total of 48 isolates and the reference strain NCIMB 14824^T of *E. piscicida* obtained from different hosts, year of isolation and geographical origin were used in this work. The antimicrobial susceptibility was assessed using the criteria described by Clinical and Laboratory Standard Institute. All isolates were tested by PCR assays to detect the presence of the genes associated with the resistance to different antibiotics.

Results: The isolates of *E. pisicicida* presented different patterns of antibiotic susceptibility but all of them were susceptible to flumequine. Antibiotic resistance genes as *arn*A and *arn*C, related with polimixin resistance; *mar*A, associated with tetracycline resistance or other implicated on beta-lactamases resistance (*amp*H) were detected.

PM280 Bioactive chitosan-silver microspheres with antimicrobial activity against recently isolated resistant bacteria

Alexandru Mihai Grumezescu[1], Alina Maria Holban[1;2], Bogdan Stefan Vasile[1], Ecaterina Andronescu[1]

[1]Department of Science and Engineering of Oxide Materials and Nanomaterials, University Politehnica of Bucharest
[2]Department of Microbiology, Faculty of Biology, University of Bucharest, Research Institute of the University of Bucharest;

Background: Antimicrobial resistance is a highly increasing phenomonon and therapeutic options are very limited. Nano and microparticles may represent a very efficient alternative in the fight of antimicrobial resistance, they being able to modulate microbial virulence, to be active against susceptible and resistant isolates and maybe to reduce resistance rates since they may have numerous unspecific microbial targets.

Objectives: The purpose of this study was to asses the antimicrobial and antibiofilm activity of some newly produced chitosan coated silver nanoparticles against antibiotic resistant Pseudomonas aeruginosa and Staphylococcus aureus clinical strains.

Methods: Silver embedded chitosan microspheres were obtained by microemulsion and physico-chemically characterized by TEM, SEM, IR and XRD. Microbial strains were isolated at the begining of 2019 form patients hospitalized in various hospitals from Romania. Quantitative antimicrobial evaluation was assesed by an adapthed disc diffusion test, while the qualitative evaluation was done through the microdilution method. Attachment was evaluated by Cravioto's modified method, while biofilm formation was evaluated by cristal violet method, microscopy analysis and viable counts.

Results: The obtained silver-chitosan spheres ranged in dimensions from 100 to 500 nm. Their shape and intrinsic antimicrobial activity of both chitosan and silver nanoparticles components made these microspheres very efficient antimicrobial agents. They proved to be highly efficient against both control, laboratory strains, but also against multidrug resistant bacteria. Moreover, their ability to reduce biofilm formation in vitro recommend these agents as promising alternatives for future antimicrobial therapy.

PM281 MALDI-TOF MS performance for Nocardia species identification

Dane Luznik[1], Viktorija Tomic[1], Vesna Spendal[1], Marusa Ravnik[1]

[1]*University Clinic of Respiratory and Allergic Diseases Golnik, Laboratory for Respiratory Microbiology, Golnik, Slovenia*

Background: Bacteria from genus *Nocardia* cause infections in immunocompromised patients. Traditionally, identification of *Nocardia spp.* was complex. Clinical laboratories had to use a combination of different methods until the introduction of MALDI-TOF MS (matrix assisted laser desorption ionization-time of flight mass spectrometry).

Objectives: Identification of *Nocardia spp.* using a combination of drug susceptibility patterns and biochemical tests was compared to identification using MALDI TOF MS biotyper.

Methods: 107 *Nocardia* isolates were used, collected between years 2009 and 2017 at University Clinic of Respiratory and Allergic Diseases Golnik. All isolates were confirmed as *Nocardia spp.* with Nocardia-specific PCR. All strains were identified using a combination of drug susceptibility patterns and biochemical tests (Kiska et al., 2002). Additionally, all strains were identified using Bruker Microflex MALDI Biotyper (Bruker Biotyper ver. 6.0.0.0, 6903 entries). High temperature extraction method of protein was performed.

Results: A combination of drug susceptibility patterns identified all strains to species level. MALDI-TOF score for species-level identification was lowered from ≤2.0 to ≤1.7. MALDI biotyper identified *Nocardia* to species level only in 42 (39%) cases. Of all *Nocardia* isolates identified by MALDI, only 18 identifications matched those obtained by combination of drug susceptibility patterns.

MALDI-TOF MS manufacturer's library does not contain sufficient number of *Nocardia spp.* spectral profiles and *Nocardia* genus has undergone great changes in taxonomy in recent years. These could be possible reasons for discrepancies between both methods as well as the combination of drug susceptibility patterns and biochemical tests being outdated.

PM282 Interspecies transfer of antimicrobial resistance genes in coagulase-negative staphylococci

Lenka Fisarova[1], Jiří Doškař[1], Nina Hulatova[1], Roman Pantůček[1]

[1]*Masaryk University, Department of Experimental Biology, Faculty of Science, Brno, Czech Republic*

Background: Coagulase-negative staphylococci (CoNS) are an important cause of human and animal diseases. Treatment of these diseases is complicated by their common antimicrobial resistance, which is often encoded by genes located on mobile genetic elements. These genes can spread throughout the bacterial population by transduction, which is the most frequent mechanism of horizontal gene transfer among staphylococci.

Objectives: The aim of this study is to characterize mobile elements in several species of CoNS, determine their similarity and antimicrobial resistance carried on them and to confirm horizontal gene transfer by transduction.

Methods: Plasmids were isolated from the analysed strains using commertial kits, antimicrobial resistance was determined by disc diffusion method and PCR. Transduction was carried out using previously described transducing phages and selection was done on agar plates with antibiotics.

Results: We analyzed plasmid content in 60 strains belonging to 10 CoNS species of human and veterinary origin and detected genes responsible for antimicrobial resistance. In total, we tested 49 transduction systems consisting of 3 different donor S. aureus strains, 2 transducing phages and 17 recipient strains belonging to 7 CoNS species. We successfully transferred plasmids encoding tetracycline and streptomycin resistance. Plasmids were transferred unchanged, were stably maintained in recipient strains, and expressed resistance genes.

Acknowledgement: The work was supported by grants 18-13064S and MUNI/A/0958/2018.

PM283 New resistance mechanism to nitrofurantoin in Escherichia coli clinical isolates

Rucha Datar[1], Valérie Chalansonnet[1], Sylvain Orenga[1], Alex Van Belkum[2]

[1]*bioMérieux, Microbiology Research, La Balme Les Grottes, France*
[2]*bioMérieux, Data Analytics, La Balme Les Grottes, France*

Background: Nitrofurantoin (NIT) is an antibiotic which belong to the class of nitrofurans and is used to treat urinary tract infections. Nitrofurantoin resistance is linked to a decrease in nitroreductase activity which has been observed in *Escherichia coli*: increased resistance due to mutations in *nfsA* and/or *nfsB* genes encoding two nitroreductases. However, the mutations in *nfsA* and *nfsB* do not fully explain the resistance to NIT by *E. coli*. Preliminary results show that mutations in the *emrR* repressor of the operon coding for the EmrAB efflux pump is involved in the resistance to nitrofurantoin.

Objectives: The aim of this work is to identify a new mechanism involved in nitrofurantoin resistance in *E. coli* to demonstrate nitrofurantoin resistance. We have compared the strains to detect mutation and have correlated mutation detection and AST profiles.

Methods: 81 clinical isolates were selected belonging to different resistance categories (non multi drug resistant=30, Multi Drug Resistant =39, Extensively Drug Resistant =12). Half of the strains were resistant to NIT and half were sensitive. The AST profiles of the *E. coli* strains were confirmed using VITEK2. Gene analysis of clinical isolates was done using PCR. Primers were designed to amplify the genes *nfsA, nfsB* and *emrR* which play a key role in developing increased resistance to nitrofurantoin.

Results: Mutations in the *emrR* repressor appear to be complementary to the resistance imparted by the *nfsA* and *nfsB* genes. They also enable very high level of resistance as they are observed in clinical strains.

PM284 Evaluation of the role of 17beta-estradiol in the response of murine macrophages inoculated with Staphylococcus aureus

Rafaela Bitencourt[1], Hanna Coelho[1], Camila Barbosa[1], Clarissa Leal[1], Manoel Neres[2], Guilherme Campos[1], Lucas Marques[1;2;3]

[1]*Universidade Federal da Bahia, Vitória da Conquista, Brazil*
[2]*Universidade Estadual de Santa Cruz, Ilhéus, Brazil*
[3]*Universidade de São Paulo, São Paulo, Brazil*

Background: Immunoendocrine evaluations are much discussed in scientific environment. Estrogen could influence the intensity of innate and adaptive immune response. However, there are no data in literature that evidence this action of estradiol in infections caused by *Staphylococcus aureus.*

Objective: To evaluate the effect of 17β-estradiol (E2) on the immune response induced by inoculation of *S. aureus* in peritoneal macrophages of male and female mice.

Methods: Females mice were used and divided ovariectomized and Sham groups. In addition, males mice were also used. The animals were sacrificed and peritoneal macrophages were isolated. Macrophages were treated with E2 and inoculated with *S. aureus*. The macrophage RNA was extracted and the gene expression of the cytokines TNF-α, IL-1β, IL-6 and IL-8 and the TLR2 receptor was performed.

Results: Increased cytokine expression was observed in all infected groups compared to their control (without *S. aureus* inoculation). In addition, the E2-treated groups showed decreased cytokine expression when compared to the untreated groups. These data may indicate an immunosuppressive activity of this hormone. Exception was observed in the expression of IL-8 in macrophages obtained from males, where the hormone behaved as immunostimulant. Studies have shown that gonadal hormones usually induce immunosuppression, and this effect may be related to the time of treatment and the dose used. Males are more responsive to infectious caused by *S. Aureus*. This responsiveness could be related to the action of the cytokine IL-8, which is known to be an important mediator of acute inflammatory response.

PM285 Bacterial Warfare: Response of the Gut Microbiota to Attaching and Effacing Pathogens

Antonio Serapio-Palacios[1], Stefanie Vogt[1], Sarah Woodward[1], Kelsey Huus[1], Madeleine Gerrie[2], Wanyin Deng[1], Hongbing Yu[3], Brett Finlay[1]

[1]*The University of British Columbia, Michael Smith Laboratories, Vancouver, Canada*
[2]*University of Waterloo, Waterloo, Canada*
[3]*The University of British Columbia, Vancouver, Canada*

Background: Diarrhea is a leading cause of illness and mortality worldwide. The attaching and effacing (A/E) pathogens are major causes of this disease, and include enterohemorrhagic *Escherichia coli* (EHEC), and *Citrobacter rodentium* (CR). The first major hurdle that A/E pathogens must overcome to infect their hosts is the gut microbiota, which is a microbial community of trillions of bacteria present in the mammalian digestive tract. To overcome these barriers, pathogens have evolved to secrete toxins directly into target cells using complex machines called secretion systems, such as the type 6 secretion system (T6SS).

Objective: Our research seeks to understand how A/E pathogens use the T6SS as a molecular weapon to attack the microbiota to cause disease.

Methods: We used an animal model of infection to identify whether the T6SS is crucial for the success of CR infection. Having determined the functionality of T6SS in CR, we measured changes of the gut microbiota composition caused by the CR T6SS using 16S rRNA gene sequencing.

Results: CR harbors two uncharacterized T6SS clusters, which we called T6SS-1 and T6SS-2. Competitive assays using T6SS-mutant strains showed that T6SS-2 mutant had a more profound disadvantage versus CR wild type. We also found T6SS-2 deletion reduces the ability of CR to colonize the colonic mucosa. Moreover, a mutant in both T6SS's attenuated CR lethality to the highly susceptible C3H/HeJ mice strain. Finally, mice feces collected at different times of infection showed that T6SS mutants lead to a different microbiota composition in comparison to CR WT.

PM286 Virulence potential of an uropathogenic Escherichia coli strain carrying the locus of enterocyte effacement (LEE)

Tiago Barcelos Valiatti[1], Ana Carolina de Mello Santos[1], Fernanda Fernandes Santos[1], Cecilia Mari Abe[2], Eneas de Carvalho[2], Rosa Silva[1], Tania Aparecida Tardelli Gomes[1]

[1]Universidade Federal de Sao Paulo, Microbiologia, Imunologia e Parasitologia, Sao Paulo, Brazil
[2]Instituto Butantan, Laboratório de Bacteriologia, Sao Paulo, Brazil

Background: Uropathogenic *Escherichia coli* (UPEC) is the foremost urinary tract infection agent. We have previously identified an UPEC strain (252) carrying the *eae* gene, which encodes the adhesin intimin and is located in the locus of enterocyte effacement (LEE) pathogenicity island in enteropathogenic (EPEC) and enterohemorrhagic (EHEC) *E. coli.*

Objectives: To analyze the pathogenic potential of UPEC 252 regarding the occurrence of diarrheagenic *E. coli* (DEC) virulence genes and their functionality.

Methods: The draft genome sequence of UPEC 252, obtained by Illumina sequencing, was analyzed regarding the presence of DEC and UPEC virulence genes, using the BLAST program and the Center for Genomic Epidemiology pipelines. Intimin production was detected by immunoblotting, bacterial adhesiveness was tested on HeLa and intestinal Caco-2 cells, and the ability to promote actin accumulation was evaluated in HeLa cells by the fluorescent actin staining (FAS) test.

Results: UPEC 252 was devoid of the *bfp* operon and *stx* genes, which encode specific virulence characteristics in EPEC and EHEC, respectively. Nonetheless, it carried a LEE region that when aligned with other LEE positive strains showed 99% identity with EHEC O157:H7 strains. UPEC 252 adhered heterogeneously on both cell lineages tested, occasionally forming clusters. Functionality of the LEE was confirmed by detection of intimin production and FAS positivity, which is an indirect demonstration of the ability to cause attaching and effacing lesion in the intestinal mucosa. Our findings suggest that such hybrid genome may allow UPEC 252 to cause intestinal and extraintestinal diseases.

PM287 Phenotypic and genotypic characterization of vancomycin-resistant enterococci isolated from patients undergoing haemodialysis

Preslava Hristova[1], Hristina Hitkova[1], Szymon Walter.de.Walthoffen[2], Mariya Sredkova[1]

[1]*Medical University Pleven, Microbiology, Virology and Medical Genetics, Pleven, Bulgaria*
[2]*Medical University of Warsaw, Medical Microbiology, Warsaw, Poland*

Background: Patients with gastrointestinal carriage are the major reservoir for vancomycin-resistant enterococci (VRE) in hospitals. Once VRE colonized patients might remain asymptomatic carriers for a long period.

Objectives: Our aim was to determine the phenotype of glycopeptide resistance, the involved genotype and the clonal relationships among VRE isolated from patients screened for intestinal colonization.

Methods: The study was performed on 27 non-repetitive VRE isolated from rectal swabs of patients undergoing hemodialysis at University Hospital-Pleven. Species identification was performed using RapID STREPT PANEL, VITEK 2 and VITEK MS. Minimum inhibitory concentrations (MICs) to nine antimicrobial agents were determined by E-test. Conventional PCR was used for detection of *vanABCDEG* in the isolated VRE. Molecular typing was conducted using SmaI-macrorestriction analysis/PFGE.

Results: In total, 5 *E. faecium*, 14 *E. gallinarum* and 7 *E. casseliflavus* were isolated. All *E. faecium* expressed high-level resistance to vancomycin (MICs>256 μg/ml) and moderate resistance to teicoplanin (MICs 4-6 μg/ml). These strains were found to carry *vanA* gene. It was revealed by PFGE that they belonged to a single genetic clone. High-level resistance to vancomycin and teicoplanin (MICs >256 μg/ml) was expressed by one *E. gallinarum* also carrying vanA gene. The other 13 *E. gallinarum* and all *E. casseliflavus* demonstrated low-level resistance to vancomycin (MICs 4-12 μg/m) and susceptibility to teicoplnin (MICs 0.5-0.75 μg/mL). PCR analysis revealed that the 13 *E. gallinarum* were carrying *vanC1* gene, while *vanC2* gene was identified in all *E. casseliflavus*. PFGE results indicated that the *vanC*-carrying enterococci belong to genetically unrelated clones.

PM288 Clinical and laboratory features of infectious exacerbations of chronic obstructive pulmonary disease

Nataliia Popenko[1], Zhanna Sobkova[2]

[1]*Ukrainian Military Medical Acadamy, Military Therapy, Kiyv, Ukraine*
[2]*National Military Madical Clinical Center, Microbiology, Kiyv, Ukraine*

Background: Chronic obstructive pulmonary disease (COPD) is one of the leading causes of death in the world. The question of antibiotic therapy role during the COPD exacerbation remained for a long time not absolutely certain.

Objectives: To examine the role of bacterial pathogens in COPD exacerbation development, to analyze diagnostic and therapeutic measures in retired military patients for the purpose of their optimization.

Methods: 72 men aged 43 to 88 years old with an infectious exacerbation of COPD were examined. To identify the etiological structure of the infectious exacerbation of COPD, data from a bacteriological study of sputum was used, which included the quantitative detection of the pathogen and its sensitivity to antibacterial drugs.

Results: The causes of exacerbations of COPD in the subjects were acute respiratory viral infections (34.7%), overcooling (15.3%), physical overload and work in unfavorable conditions (5.5%), decompensation of concomitant pathology (5.5%). In 40% of cases, the cause of the exacerbation was unknown.
Types 1 (44.5%) and 2 (43%) exacerbations were most often observed in the examined patients according to Anthonisen, and type 3 - only in 12.5% of cases.
It was found that representatives of the family of Streptococcus (S. pneumoniae, S. mitis, S. viridans, S.epidermidis) caused infectious exacerbations of COPD in 53.3% of the patients, Staphylococcus - in 16.6%, Ps . auruginosa - in 6.6%.
Most pathogens were susceptible to ß-lactam antibiotics, namely amoxicillin / clavulanate (95.8 ± 8.5) % and ceftriaxone (95.8 ± 8.5) %.

PM289 Polyhexamethylene guanidine salts as an effective biofouling and DNA inhibitor for polymers and protective coatings

Olena Moshynets[1], Kris De Baere[2], Raf Meskens[2], Max Leyn[2], Jean-François Bardeau[3], Oksana Tarasyuk[4], Stanislav Makhno[5], Tetiana Cherniavska[5], Oleg Dzhuzha[4], Geert Potters[2], Sergiy Rogalsky[4]

[1]*Institute of Molecular Biology and Genetics of National Academy of Sciences of Ukraine, Kiev, Ukraine*
[2]*Antwerp Maritime Academy*
[3]*Institut des Molécules et Matériaux du Mans*
[4]*V. P. Kukhar Institute of Bioorganic Chemistry and Petrochemistry of NAS of Ukraine*
[5]*Chuiko Institute of Surface Chemistry of NAS of Ukraine*

Background: The bacterial capability to form biofilms contributes to many unwanted processes, from the corrosion and the biofouling of industrial installations, ships, ports, to the spread of hospital acquired catheter-related infections and antibiotic resistance. A new, effective, non-leaching agent for inclusion in polymers like paints and plastics could reduce biofilm development.

Objectives: To investigate the antifouling characteristics of new polyhexamethylene guanidine (PHMG)-based polymers in two practical applications: ship's hull epoxy-based paint and the medical engineering thermoplastic polyamide 11 (PA11).

Methods: chemical synthesis, FTIR, spectrophotometry, biofilm assays, MTT assays, statistics.

Results: In this study, a hybrid organic/inorganic biocide PHMG 2-mercaptobenzothiazolate/montmorillonite (PHMG-MMT) was synthesized. Metal coupons were covered with epoxy-based paint containing 5 wt% of the new biocide. The samples were exposed to seawater at the Ostend port for three months. It has been established that PHMG-MMT affected the development of the fouling layer on the coating surface. Another hydrophobic polymeric biocide, PHMG 2-naphtalenesulfonate (PHMG-NS) was synthesised and used as antimicrobial additive for polyamide 11 (PA-11). In biofilm assays, a substantial decrease in biofilm metabolic activity and biofilm biomass was observed for PA-11 films containing 7% and 10% of PHMG-NS for two opportunistic bacteria, *E. coli* K12 and *S. aureus* ATCC 25923. The release of eDNA into the culture medium was substantially reduced suggesting that at least one mechanism of antibiofilm activity of PHMG-NS-containing PA-11 is associated with an eDNA reduction, affecting the initial stages of bacterial biofilm formation and, thus, decreasing the spread of antibiotic resistance genes in a hospital-related environment.

PM290 Typing of Treponema pallidum subsp. pallidum in the Czech Republic during 2004-2017: clinical isolates belong to 25 allelic profiles

Eliška Vrbová[1], Markéta Nováková[1], Juraj Bosák[1], Linda Grillová[1], Petra Pospíšilová[1], David Smajs[1]

[1]*Faculty of Medicine, Masaryk University, Department of Biology, Brno, Czech Republic*

Background: While syphilis diagnosis is mainly based on anamnestic data, clinical findings and results of serological tests, PCR detection and molecular typing helps to diagnose syphilis, especially in the cases with negative serology.

Objectives: The main aim of this study was to analyze swab and whole blood samples from patients suspected of having syphilis. The detected treponemal DNA was then subsequently used for molecular typing of syphilis treponemes.

Methods: A Multilocus Sequence Typing scheme for *Treponema pallidum* subsp. *pallidum* was applied to clinical samples collected from 2004 to 2017 from the two largest cities (Prague and Brno) in the Czech Republic. Altogether, a total of 675 samples was tested in this study, 369 of them were found PCR-positive for treponemal DNA and 281 PCR-positive samples were typeable.

Results: Out of 281 typeable samples, 136 were fully-typed in all TP0136, TP0548 and TP0705 loci. Among the fully and partially typed samples, 25 different allelic profiles were identified. Distribution of TPA allelic profiles identified in the Czech Republic from 2004 to 2017 revealed a dynamic character with allelic profiles disappearing and emerging over time. While an increasing number of samples with A2058G mutations (causing macrolide resistance) was detected, the number of samples harboring A2059G mutations was found to decrease over time. Moreover, several associations of allelic profiles with age of patients, gender of patients as well as location, where patients were living, were found.

PM291 Molecular epidemiology of oxacillinases among clinical isolates of Acinetobacter spp. in Serbia

Bojana Lukovic[1], Ina Gajic[2], Sanja Zornic[3], Tatjana Pozder[4], Anita Sente Zigmanovic[4], Snezana Delic[5], Svetlana Radisavljevic[6], LJiljana Radosavljevic[6], Dejan Lazic[7], Natasa Opavski[2], Lazar Ranin[2]

[1]*College of professional health studies in Belgrade*
[2]*Institute of Microbiology and Immunology, Medical Faculty, Universty of Belgrade*
[3]*Clinical Center Kragujevac, Department of Microbiology*
[4]*General hospital Subotica, Depatment of Microbiology*
[5]*Institute of Public Health Sombor, Center of Microbiology*
[6]*General hospital Pancevo, Department of Microbiology*
[7]*Clinical Center Kragujevac, Clinic of General and Thoracic Surgery*

Background: The increasing trend of carbapenem resistance (CR) in *Acinetobacter spp.*worldwide limits drastically therapeutic options. Oxacillinases (OXA enzymes), divided into 4 phylogenetic subgroups: OXA-51-like, OXA-23-like, OXA-24-like and OXA-58-like have emerged globally as the main mechanism responsible for CR.

Objectives: The aim of this study was to investigate antimicrobial susceptibility pattern and to detect OXA encoding genes in clinical isolates of *Acinetobacter spp.* in Serbia.

Methods: From July 2018 to December 2018, 150 non−repetitive *Acinetobacter spp.* isolates recovered from patients hospitalized at 7 hospitals in Serbia, were received from clinical microbiology laboratories. The strains were identified using phenotypic tests and detection of *bla*OXA-51-like gene. Susceptibility testing was done by VITEK 2 System and broth microdilution method. All isolates were subjected to multiplex PCR for detection of *bla*OXA-51-like, *bla*OXA-23-like, *bla*OXA-40-like and *bla*OXA-58-like genes.

Results: Majority of isolates were from surgical wards-54.7% and intensive care units-37.3%. Most prevalent samples were wounds exudates-41.3% and tracheal aspirates-24%. A total of 138 *Acinetobacter spp.* strains were determined as multidrug-resistant. The rates of resistance to meropenem, imipenem, amikacin, gentamicin, tobramicin, ciprofloxacin, levofloxacin, trimethoprim-sulfamethoxazole and colistin were 92%, 92%, 92%, 92.7%, 67.3%, 96.7%, 96.7%, 89.33% and 0%, respectively.
Among 118 isolates that harbored a *bla*OXA-51-like gene, 26 strains carried a *bla*OXA-51-like gene only , 46 strains carried both *bla*OXA-51-like and *bla*OXA-23-like genes, 46 strains carried both *bla*OXA-51-like and *bla*OXA-24-like genes and not one isolate carried *bla*OXA-58-like gene. As for the rest 32 *bla*OXA-51-like negative isolates, 8 strains carried *bla*OXA-24-like gene and 4 strains carried *bla*OXA-23-like gene.

PM292 Analysis of biofilm phenotypes of 350 Stenotrophomonas maltophilia clinical isolates reveals high levels of phenotypic and structural heterogeneity

Ifey Alio[1;2], Mirja Gudzuhn[1], Christel Vollstedt[1], Uwe Mamat[3], Ulrich Schaible[4], Thomas Kohl[5], Stefan Niemann[5], Jörg Steinmann[6], Wolfgang Streit[1]

[1]*University of Hamburg, Microbiology and biotechnology, Hamburg,*
[2]*University of Hamburg, microbiology and biotechnology, Hamburg,*
[3]*Research Center Borstel, Cellular Microbiology, priority area infections, Borstel,*
[4]*Research Center Borstel, Cellular Microbiology, priority area infections, Borstel, Germany*
[5]*Research Center Borstel, German Center for infection research TTU-TB, Borstel,*
[6]*Institute for clinical hygiene, Medical microbiology and infection, Nurnberg,*

Background: Biofilm-producing bacteria are a major cause of morbidity and mortality in immunocompromised patients. The multidrug resistant opportunistic pathogen Stenotrophomonas maltophilia is today in clinical settings a relevant pathogen that can contribute significantly to disease progression in these patients.

Objectives: It is our intention to generate a very large and global data set on *S. maltophilia* and its biofilm formation on a genus and pangenome-wide level. We are currently analyzing the genome of over 350 clinical and 40 environmental isolates. Up to date we have established the genome sequences of over 100 isolates (Steinmann, Front Microbiol. 2018; 9: 806) and the genome sequencing of additional 250 isolates is ongoing. In parallel we are investigating the biofilm profiles of these isolates and correlating this with transcriptome data.

Methods: Genomes of over a 100 isolates were sequenced using NGS technologies and a phylogenetic tree with 12 sub-groups was constructed (Steinmann, Front Microbiol. 2018; 9: 806). Biofilm assays done in microtiter plates and in flow cells revealed a strong variation in biofilm forming ability and biofilm structures among the isolates. This high level of heterogeneity was independent of the strains and their phylogenetic position within the genus.

Results: *S. maltophilia* isolates both clinical and environmental display a diverse biofilm phenotype. Because of these observations, we speculate that under *in vivo* conditions *S. maltophlilia* displays varying biofilm architectures on a strain-specific level. The strains specific heterogeneity may just be another strategy to escape antibiotic treatment or host immune defense.

PM293 Detection and identification of Mycobacterium bovis in cattle in Bulgaria

Tanya Savova-Lalkovska[1], Violeta Valcheva[2], Albena Dimitrova[1], Hristo Najdenski[2], Magdalena Bonovska[2]

[1]*National Diagnostic and Research Veterinary Medical Institute, Sofia, Bulgaria*
[2]*The Stephan Angeloff Institute of microbiology, BAS, Sofia, Bulgaria*

Background: Bovine tuberculosis is still a serious problem for many countries including Bulgaria. The increased transport of animals requires permanent monitoring of the disease and will help the risk assessment for humans. Timely detection of the vectors of infection and identification of the factors contributing to transmission of *M. bovis* are important to control the disease.

Objectives: Detection and identification of of *Mycobacterium bovis* in diagnostic materials from cattle, reacted positive or doubtful to PPD tuberculin.

Methods: Diagnostic materials from lymph nodes from cattle during the last two years were studied. Application of pathomorphological, microbiological and PCR methods were used for the identification of mycobacterial isolates. Bacterial cultivation was carried out by Löwenstein–Jensen agar and Stonebrink medium. The detection of *M. bovis* was done by conventional PCR. RD4 -PCR was used as a specific reaction for detection of RD4 deletions to confirm the *M. bovis* in the MTB complex.

Results: Bacteriologically were isolated 220 *M. bovis* isolates from the investigated 628 altered lymph nodes. Wet, slimy whitish colonies of mycobacteria were observed in all isolated strains on solid medium after 4-8 weeks. The IS6110 specific for *M. bovis* was observed in all isolates. The distribution of RDs allowed us to differentiate the isolates among the tested *M. bovis* strains. The most common, showed the greatest number of RDs and seem to have undergone the greatest loss of DNA relative to other members of the MTB complex.

Acknowledgements: This work was supported by DN16/12 of the NSF, Bulgaria.

PM294 Virulence genotypes of Avian Escherichia coli strains, isolated from suspected broiler to colibacillosis in Iran

Ali Goudarztalejerdi[1], Sajad Najafi[1], Farangis Nargesi[1], Abdolmajid Mohammadzadeh [1], Amir Hossein Babaeipour[2]

[1]*Hamadan Province, Faculty of Veterinary Science, Bu-Ali Sina University, Department of Pathobiology, Iran*
[2]*Private practitioner, Amir Makian Hekmatneh Veterinary Services Company, Toyserkan, Iran*

Background: Avian Colibacillosis is a complex syndrome that causes great economic loss in the poultry industry. Avian Pathogenic *Escherichia coli* (APEC) is the most considered aetiological agent of avian Colibacillosis. The pathogenisity of APEC associated with serogroups, and virulence factors coded by virulence associated genes (VAG). Virulence factors are involved in colonization, adhesion, invasion, and survivial of E.coli against host defenses. Among these factors, the *fimC* (fimberiae mannose-binding type1), *cvaA/B* (Colicin V A/B), *iutA* (aerobactinsiderophore receptor), *Iss* (Increased serum survival) and *SitA* (a bacterial iron transporter) genes have important roles in the APEC pathogenesis.

Objectives: The aim of this study was to determine the virulence genotype of Avian *E. coli*, isolated from suspected broiler to colibacillosis in broiler farms in the Hamedan, West of Iran.

Methods: The present study was carried out on *E.coli* strains (n=100), isolated from visceral organs of diseased and dead poultry suspected to colibacillosis from different broiler flocks. The presence of five VAG in APEC's including *FimC*, *cvaA/B*, *iutA*, *Iss* and, *SitA* were investigated using multiplex PCR.

Results: The multiplex PCR analysis showed that the *iutA*, *fimC*, *Iss*, *SitA* and *cvaA/B* virulence associated genes had detection rates of 97%, 85%, 84%, 82%, and 52% respectively. Thirty-three of 100 isolates (33%) harbored five VAG which are known as APEC, and one isolate contains only one VAG.

PM295 Carvacrol is a potent inhibitor of vibrio cholerae pathogenesis

Nabendu Sekhar Chatterjee[1], Suman Das[1], Keinosuke Okamoto[2], Asish Mukhopadhyay[3]

[1]*ICMR-National Institute of Cholera and Enteric Diseases, Biochemsitry, Kolkata, India*
[2]*Collaborative Research Center of Okayama University for Infectious Diseases at NICED, Kolkata, India*
[3]*ICMR-National Institute of Cholera and Enteric Diseases, Bacteriology, Kolkata, India*

Background: Novel antibacterials are urgently needed to address growing problem of bacterial resistance to conventional antibiotics. Essential oils possess important volatile compounds with diverse bioactivities including antimicrobial potential.

Objectives: Here we have studied the role of carvacrol, a naturally occurring essential oil fraction of Oregano (Origanum vulgare) on Vibrio cholerae pathogenesis.

Methods: We followed CLSI protocol to determine the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Carvacrol. Effect of carvacrol was investigated on mucin penetrating ability, diarrheal cholera toxin production, virulence gene expression, adhesion to intestinal cell-line and biofilm formation.

Results: In our study, MIC and MBC of Carvacrol on V. cholerae growth is 250 µg/ml. V. cholerae motility in Carvacrol treated 1.5% mucin colum was 9 fold less defective than the untreated control. Carvacrol treated ex-vivo adhesion to HT-29 cell line reduced significantly at one-fourth MIC. Quantitative RT-PCR assay revealed that Carvacrol down-regulated the transcription of tcpA, toxT and ctxB genes at one-fourth MIC. At one-eighth MIC Carvacrol completely inhibits the formation of biofilm. These results suggest that carvacrol might act as potent inhibitor of virulence, adhesion and motility which ultimately reduces the pathogenicity of V. cholerae. It can be concluded that carvacrol can be added to food products at doses below the MIC value, thereby reducing the risk of adhesion and toxin production by V. cholerae and increasing the safety of the products.

PM296 Molecular insights on SaPI inducing mechanism

Mohammed Alqasmi[1], Julio Bacarizo[1], Alberto Marina[2], Jose Penades[1]

[1]*University of Glasgow, College of Medical, Veterinary and Life Sciences, Glasgow, United Kingdom*
[2]*Instituto de Biomedicina de Valencia, València, Spain*

Background: *S. aureus* pathogenicity islands (SaPIs) are parasitic mobile genetic elements that exploit phages for induction and transfer. SaPIs integrate to the host chromosome and are repressed by Stl, the SaPI master regulator. SaPI induction is occurred when the SaPI Stl/DNA complex is disrupted via a specific protein encoded by helper phage. The inducer for SaPI1 is a phage protein called Sri. Interestingly, and in addition to SaPI1 de-repression, Sri blocks bacterial DNA replication by binding to the helicase loader protein (DnaI).

Objectives: The fact that a small protein like Sri (52aa) interacts with two unrelated proteins raises several interesting questions; are DnaI and Stl sharing similar structural conformations being recognized by the same interacting residues on Sri, or by contrast, Sri has two interacting faces, one to interact with DnaI and the other to interact with Stl? Unraveling those questions would define SaPIs nature. If the DnaI and Stl share a conserved domain or similar fold, SaPIs would be then considered as phage parasites. By contrast, if Sri has two different interacting regions, this would imply in somehow that SaPIs can provide unrecognized advantages for the phage.

Methods: To solve the above questions, *in vivo* and *in vitro* methods have been used including molecular, biochemical and protein crystallography techniques.

Results: Our results provide insights on the mechanism that is used by SaPI1 to interact with Sri protein, highlighting these elements as one of the most fascinating mobiles genetic elements in nature.

PM297 Poly-microbial biofilm interaction by Histophilus somni and Pasteurella multocida in vitro and in vivo, and their effect on host response

Thomas Inzana[1], Briana Petruzzi[2], Allan Dickerman[3]

[1]*Long Island University, College of Veterinary Medicine, Brookville, United States*
[2]*Virginia Tech, College of Veterinary Medicine, Blacksburg, United States*
[3]*Virginia Tech, Biocomplexity Institute, Blacksburg, United States*

Background: *Histophilus somni* and *Pasteurella multocida* cause bacterial bovine respiratory disease (BRD). Both species form a biofilm, individually and together, *in vitro* and *in vivo*. However, how these two species interact with each other and their host in a poly-microbial biofilm is unknown.

Objectives: Our objectives were to determine how *H. somni* and *P. multocida* intermix and effect gene expression in a poly-microbial biofilm *in vitro* and in the natural host.

Methods: The interaction of *H. somni* and *P. multocida* cells and biofilm matrix was examined by fluorescence *in situ* hybridization and fluorescently tagged lectins to the matrix exopolysaccharide (EPS), respectively, by confocal scanning laser microscopy. The gene expression of each bacterial species and the host was examined by RNA-sequencing.

Results: Bacterial cells and matrix material of both species were evenly distributed in the biofilm. However, a poly-microbial biofilm could only be established if *P. multocida* was added to an established (3-day old) *H. somni* biofilm. About half the *H. somni* genome was differentially regulated when the bacteria were grown as a biofilm, compared to planktonic growth, and 282 *P. multocida* genes were significantly differentially regulated when grown as a poly-microbial biofilm. At least 93 host genes, many associated with inflammation, were upregulated $\geq$ 2.5 fold at the site of infection. In BRD samples from which both *H. somni* and *P. multocida* were isolated only the EPS of *H. somni* was detected with fluorescently-tagged lectins, likely because *P. multocida* EPS is not expressed when the bacteria are encapsulated *in vivo*.

PM298 Comprehensive assessment of the ability of non-typhoidal Salmonella and Enterococcus faecium to survive acidic environments

Andreia Rebelo[1;2], Joana Vanessa Cordeiro Melro Mourão[1], Ana Raquel Freitas[1], Joana Campos[1], Luisa Maria Vieira Peixe[1], Patricia Antunes[1;3], Carla Novais[1]

[1]UCIBIO/REQUIMTE, Department of Biological Sciences, Microbiology Laboratory, Faculty of Pharmacy, University of Porto, Portugal
[2]School of Health, Polytechnic of Porto, Portugal
[3]Faculty of Nutrition and Food Science, University of Porto, Porto, Portugal

Background: Acidic-pH resulting from acid-products use or naturally occurring in different environments (e.g.food-production-chain-feed/disinfectants/human/animal hosts) potentially selects multidrug-resistant (MDR)-bacterial strains. Remains unknown if particular MDR-strains of pathogenic bacteria are more prone to overcome acid-stress.

Objectives: To assess susceptibility to acidic pH of non-typhoidal *Salmonella* and *Enterococcus faecium* with diverse epidemiological and genetic backgrounds.

Methods: We included 45 *E.faecium* (clades:A1-n=12/A2-n=18/B-n=6/not-identified-n=9/82%-MDR) and 56 *Salmonella* (*S.*4,[5],12:i:-n=17; *S.*Typhimurium-n=6; *S.*Enteritidis-n=3; 10 other serotypes-n=30/79%-MDR) from humans (healthy/clinical-n=39) and animal-production-setting/foods (n=62) (1997-2018/6-countries). The minimum-growth-pH (growth-pH_{min}) was assessed by broth-microdilution using Mueller-Hinton-II adjusted with HCl (pH=2.0-6.5/16h-20h±2h/37ºC) and the minimum-survival-pH (survival-pH_{min}) by plating microdilution wells without visible growth in Mueller-Hinton-II-agar (24h-48h±2h/37ºC). An Acid-Tolerance-Response-assay (ATR; HCl-shock-treatment-pH=3.0/15´-*Salmonella*/60'-*E.faecium*; or HCl-pre-adaptation-pH=4.5/60'+shock-treatment-pH=3.0/15´-*Salmonella*/60'-*E.faecium*) was performed in 3 isolates/each-genera (different growth/survival-$pH_{min,}$) followed by growth-pH_{min} and survival-pH_{min} assays.

Results: The growth-pH_{min} for most *Salmonella* was 4.0 (98%-n=55/56) and the survival-pH_{min}=3.5 (52%-n=29/56) or 4.0 (48%-n=27/56). For *E.faecium* the growth-pH_{min} was 4.5 (51%-n=23/45) or 5.0 (49%-n=22/45) and the survival-pH_{min} was 3.0 (18%-n=8/45), 3.5 (40%-n=18/45) or 4.0 (42%-n=19/45). The ATR-assay with pre-adaptation+shock-treatment enhanced survival-pH_{min} from 3 to 2.5 in *E.faecium* (n=1) and from 4 to 3.5 in *S.*4,[5],12:i:- (n=1). At survival-pH_{min}=3.5-4.0 isolates from different origins, serotypes-*Salmonella* or clades-*E.faecium* were detected. At survival-pH_{min}=3.0 only *E.faecium* (n=8/all-MDR; including the one with improved survival in ATR-assay), from a poultry-processing-plant using peracetic-acid as disinfectant, survived. Our data suggest that MDR *Salmonella* and *E.faecium* with diverse epidemiological and genetic backgrounds can survive to low-pH values found in diverse environments/hosts, although differences among clades/serotypes were not detected. ATR-assays revealed strain-specific ability to adapt to middle HCl-pH=4.5.

PM299 Expression and characterization of bacteriocins among Shiga toxin-producing Escherichia coli

Caio Antonio Marcondes[1], Evanilde M Gonçalves[1], Beatriz E C Guth[1]

[1]*Universidade Federal de Sao Paulo, Microbiology, Immunology, Parasitology, São Paulo, Brazil*

Background: Shiga toxin-producing *Escherichia coli* (STEC) are important food-borne pathogens. Production of bacteriocins may contribute to the ability of *E. coli* strains to competitively dominate some niches.

Objectives: Investigate the production of bacteriocins among STEC strains of different serotypes isolated from human infections, from the animal reservoir and the environment.

Methods: A total of 251 STEC strains from diverse origins was evaluated for bacteriocin production against a sensitive indicator strain. The sequences for *col Ia / Ib*, *col Ia*, *col Ib*, *col E2* and *col V* genes was searched by PCR.

Results: Expression of bacteriocins was identified in 62% of STEC strains. Higher frequencies were observed among STEC of human origin (91%) and isolates from water (100%), and ranged from 48% to 100% according to the animal species. Production of bacteriocins was identified in a wide variety of serotypes, and 49% of the strains carried some of the bacteriocin genetic sequences studied. Genes for *colIb* (59%) followed by *colIa / Ib* (19%) and *colE2* (16%) were those identified. Conclusion: A high frequency of bacteriocin expression was identified among STEC strains of human origin, animal species and the environment in Brazil. Despite the diversity of serotypes identified among bacteriocinogenic strains, the frequency of bacteriocins in STEC serotypes responsible for causing severe human infections could indicate its participation as a virulence factor. In addition, the high occurrence of bacteriocins in STEC strains isolated from different animal species and the environment may suggests its involvement as a mechanism of persistence in these reservoirs.

PM300 Kodamaea ohmeri, an emerging fungal pathogen- a case report and review

Shin-Yi Tsai[1;2], Po- Ya Tung[1], Yu-Ting Tina Wang[1], An-Chun Kao[1], Chien-Feng Kao[3]

[1]*Mackay Memorial Hospital, Department of Laboratory Medicine, Taipei City, Taiwan*
[2]*Mackay Medical College, Department of Medicine, New Taipei City, Taiwan*
[3]*Mackay Memorial Hospital, Department of Infectious Disease, Taipei City, Taiwan*

Background: *Kodamaea ohmeri* (*K. ohmeri*) is a rare yeast-like fungus, but can be potentially life-threatening when its infection is a cause for severe sepsis. Its infection leads to a high mortality rate, as immunocompromised patients are susceptible to this opportunistic pathogen.

Objectives: Early diagnosis and treatment for this pathogen is critical in managing potentially fatal infections.

Methods: A 63-year-old man with a history of heart disease and gastric cancer with liver metastasis visited the Emergency Department of Mackay Memorial Hospital complaining of abdominal fullness, poor appetite and weight loss of 20kg in three months.

The initial laboratory data showed elevated white blood cell count 13,300 cells/μl (reference range 4000-10000), with segmented neutrophil: 86.3% and lymphocyte: 5.2%. C-reactive protein (CRP) was elevated at 3.272mg/dL (reference 0-0.79). Chest X-ray revealed pleural effusion or pneumonia over right middle lobe. An initial set of blood culture was drawn.

After three days the initial set of blood culture yielded a yeast growth. Five days after admission, the blood culture was positive for *Kodamaea ohmeri*.

Results: Diagnosing *K. ohmeri* is mainly made by blood culture. Commercial assays such as API 20C system and Vitek 2 ID YST system for identification are regularly used. However, misidentification with Candida species has been reported.

Currently no set guidelines exist, but treatments may consist of removing any artificial implantations and selecting appropriate antifungal medications, noting resistance to Fluconazole.

Raising awareness, developing more accurate methods to early recognition, and timely treatment of this pathogen would be crucial in high risk patients.

PM301 The action of Candida glabrata cell wall-bound proteases-Yps3 and Cwp1-on the human complement system

Dorota Satala[1], Marcin Zawrotniak[2], Aleksandra Zelazna[1], Andrzej Kozik[1]

[1]Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University in Krakow, Analytical Biochemistry, Krakow, Poland
[2]Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University in Krakow, Comparative Biochemistry and Bioanalytics, Krakow, Poland

Background: *Candida glabrata*, a yeast-like fungus closely related to non-pathogenic *S. cerevisiae* yeast, is currently the second, after *C. albicans*, most common causative agent of human candidiasis. Its pathogenicity depends on the production of multiple virulence factors, one of the most important being a set of extracellular (mostly cell-wall bound) proteases that can help the pathogen to evade the host defense mechanisms. So far, *C. glabrata* genes that encode 11 aspartic proteases—yapsins (Yps)—and one serine protease—Cpw1—have been identified.

Objectives: The aim of this study was to purify two extracellular proteases of *C. glabrata*—Yps3 and Cwp1—and to examine their possible inhibitory role in the activation of the host complement system.

Methods: The ion-exchange chromatography and high-performance gel filtration were used to purify *C. glabrata* surface-exposed Yps3 and Cwp1. The degradation of complement components C3 and C5 by the yeast proteases was analyzed using SDS-PAGE. The interaction of *C. glabrata* proteases with C3 and C5 was detected by a microplate ligand-binding assay. The influences of Yps3 and Cwp1 on C3b surface deposition and C5a generation were determined by flow cytometry and ELISA assay, respectively.

Results: Yps3 and Cwp1 proteases are secreted by *C. glabrata* and deposited on the cell surface. Both proteases bind and cleave human complement components C3 and C5 at neutral pH. Yps3 prominently inactivates the central complement components C3b and C5a, thus reducing the pathogen-damaging potential of the activated complement system.

This work was supported by the Jagiellonian University (Statutory Funds of the FBBB BMN 13/2018 awarded to DS).

PM302 Isolation of Rickettsia raoultii from Dermacentor reticulatus ticks from Austria

Michiel Wijnveld[1], Anna-Margarita Schoetta[1], Adriano Pintér[2], Gerold Stanek[1]

[1]Medical University of Vienna, Institute for Hygiene and Applied Immunology, Vienna, Austria
[2]Public Health Department, Superintendência de Controle de Endemias, São Paulo, Brazil

Background: With the emergence of newly discovered pathogens transmitted by ticks, new or optimised culture techniques are required to study these microbes under laboratory conditions. The success of the cultivation of intracellular microorganisms largely depends on the suitability of cell-lines employed. During this study we implemented a culturing procedure using tick cells (BME/CTVM2).

Objectives: The presented study was designed to assess the viability of tick cells, originating from *Rhipicephalus microplus,* to isolate and propagate *Rickettsia raoultii* from Austrian *Dermacentor reticulatus* ticks.

Methods: *Dermacentor reticulatus* ticks were collected by hand from the vegetation in the Donau-Auen (Lobau) national park in Vienna, Austria. Of these collected ticks, ten were selected and a leg was cut from each tick to screen the haemolymph for the presence of Rickettsia raoultii DNA by PCR-reverse line blot hybridization. A single tick tested positive. The positive tick was surface disinfected, homogenised and aliquots of the homogenate was used to infect *Rhipicephalus microplus* BME/CTVM2 cells.

Results: Sixty-five days after the inoculation with the tick extract, we observed *Rickettsia*-like intracellular bacteria in the cultured cells. From the cultures, DNA was extracted and *R. raoultii* presence was confirmed by PCR followed by sequencing the 16S ribosomal RNA, *sca4*, *ompA*, *ompB* and *gltA* genes in addition to the 23S–5S ribosomal RNA intergenic spacer region. Obtained sequences were compared using NCBI BLAST and showed 99 – 100 % similarity to other available *R. raoultii* strains.

PM303 Characterization of Listeria monocytogenes isolates from human clinical episodes ocurring from 2013 to 2018

Sofia Araújo Pereira[1], Vânia Ferreira[1], Paula Teixeira[1]

[1]*Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Porto, Portugal*

Background: *Listeria monocytogenes* is a major foodborne pathogen in humans, responsible for listeriosis, a rare but life-threatening systemic infection among susceptible population groups: the elderly, immunocompromised individuals, pregnant women and new-borns. The consumption of contaminated food is recognized as the main transmission route to humans, and population aged over 65 accounts for most reported listeriosis cases.

Objectives: This work aims to obtain epidemiological data on human listeriosis cases, collected from collaborating hospitals between 2013 and 2018.

Methods: *L. monocytogenes* isolates were collected from volunteer hospitals and were characterized based on genoserotyping by multiplex polymerase chain reaction and on DNA macrorestriction pulsed-field gel electrophoresis (PFGE), applying the enzymes *Asc*I and *Apa*I.

Results: Over the study period, 129 isolates of *L. monocytogenes* were recovered from human cases of listeriosis. Six cases (4.7%) corresponded to maternal/neonatal (MN) infections, covering 3 fetal losses or neonatal deaths. From the information available, 91 cases were confirmed nonmaternal/neonatal (non-MN); 54.9% occurred in patients aged over 65 years and at least 10 cases (11.0%) resulted in death.

L. monocytogenes isolates belonging to genoserogroup IVb (serotypes 4b, 4d, and 4e) were responsible for the majority of listeriosis cases (73.6%), followed by genoserogroup IIa (serotypes 1/2a and 3a) isolates (16.3%), genoserogroup IIb (serotypes 1/2b, 3b, and 7) isolates (7.0%) and genoserogroup IIc (serotypes 1/2c and 3c) isolates (3.1%). PFGE analysis revealed a high molecular diversity but notwithstanding, several isolates from different geographic and time distributions were grouped into major clusters, given the close PFGE-related types among them.

PM304 Improvement of chemical fixation effect by high oxygen-containing water -Cytological analysis of Ralstonia solanacearum-

Hidefumi Maeda[1]

[1]*Faculty of Science and Technology, Ryukoku University, Department of Mechanical and Systems Engineering, Ootsu, Shiga, Japan*

Background: *Ralstonia solanacearum*, soil pathogen bacterium, causes wilting symptoms in tomato plants. To understand the tomato root infection strategy of *R. solanacearum*, cytological analysis by transmission electron microscope is important. However, the sample prepared by using the conventional chemical fixation method has insufficient protein fixation by glutaraldehyde and therefore has low cell structure preservation. Consequently, it was difficult to judge the cellular responses between *R. solanacearum* and tomato roots at each stage of infection.

Objectives: We focused on the reaction mechanism of glutaraldehyde and amino group of protein, which requires a large amount of oxygen, and investigated whether protein fixation is improved by adding oxygen to the sample.

Methods: Chemical fixation of samples were used glutaraldehyde dissolved by high oxygen-containing water which is thought to have good permeability into the inside of the cell. In order to investigate the protein fixation effect of these samples, sections cut out from the resin block produced by the chemical fixation method were analyzed histologically and cytologically.

Results: We revealed that the dissolved oxygen absorption rate in the case of immersing the sample in oxygen water is significantly higher than that in the case of distilled water. The results of histlogical and cytological analysis indicated that high oxigen-containing water could improve protein fixation effect of *R. solanacearum* and tomato roots.

PM305 Cytological analysis on morphological differentiation of Ralstonia solanacearum during the infection process

Kanako Inoue[1], Yasufumi Ibuchi[2], Yasufumi Hikichi[3]

[1]Osaka University, Graduate School of Engineering, Osaka, Japan
[2]Osaka University, Research Center for Ultra-High Voltage Electron Microscopy, Osaka, Japan
[3]Kochi University, Faculty of Agriculture and Marine Science, Department of Agricultural Chemistry, Kochi, Japan

Background: *Ralstonia solanacearum* is a bacterium that infects from roots of about 200 various kinds of economically important agricultural crops such as tomatoes, potatoes, eggplants, bananas and ginger etc. and causes wilting symptoms, which leads to a significant reduction in harvesting rate.

Objectives: Although measures to control are urgently required, there are still many ambiguous phenomena concerning morphological differentiation in the infection process. The reason for this is that it is difficult to reproduce in vitro the morphogenesis induced in plant cells and analyze cytologically.

Methods: In order to induce the differentiation of *R. solanacearum* in vitro, we made Au-C discs as a culture plate. And to ascertain wheter *R. solanacearum* incubated on the Au-C discs produce the infection structures, time-lapsed analysis by electron microscope was carried out.

Results: In this study, we have successfully reproduced in vitro the morphological differentiation similar to that in the intercellular spaces of plants by preparing Au-C discs. Time-lapse EM observation of the bacteria cultured on those disks, it was revealed that cytologically captured the maturation process from micro colony formation to mushroom type biofilm and mutual recognition between bacteria, and we succeeded in monitoring variations of elements such as carbon, oxygen, phosphorus, calcium and potassium which are thought to be associated with activity. Based on these results, it is expected that very useful information can be quickly obtained in the future for constructing the model of infection pattern and pathogenicity mechanism of R. solanacearum.

PM306 Functional analysis of ORF2271/ORF2272 in Staphylococcus aureus

Nian-Jen Li[1], Mei-Hui Lin[1]

[1]*Chang Gung University, Department of Medical Biotechnology and Laboratory Science, Tao-Yuan, Taiwan*

Background: In a previous screening work, a mutant JM7 that grows slowly and has a transposon insertion at gene *orf2271* of *Staphylococcus aureus* HG001 was selected for further study. Sequence analysis revealed that *orf 2271* encodes a protein with unknown function. The *orf2271* and its adjacent *orf 2272,* which encodes a transmembrane protein, is likely to be an operon. For investigating the functions of the operon, *orf 2271* and *orf 2271/orf2272* were knocked out to generate JM8 (single knockout) and JM9 (double knockout).

Objectives: Investigating the characteristics and functions of *orf 2271/orf2272* in *S. aureus*.

Methods: RT-PCR were used to elucidate the expression of *orf 2271* and *orf2272* in different growth conditions. DNA microarray analysis was performed to identify the genes affected by *orf 2271/orf2272.* The roles of *orf 2271/orf2272* in pathogenesis were evaluated using *Galleria mellonella* larvae infection and mice infection models.

Results: The results showed that the expression of *orf 2271* and *orf2272* reached a maximum level at exponential growth phase. Deletions in *orf2271* and *orf2272* increased expression of *vraX* and virulence-related genes including *hld*, *psmβ1* and *psmβ2*. In the model of *Galleria* larvae infection, the survival rates of larvae infected with the JM8 and JM9 were lower than that infected with the wild type strain. The similar results were also observed in a mice infection model, indicating that the novel operon including *orf2271* and *orf2272* plays a role in modulating gene expression and involved in the pathogenesis of *S. aureus*.

PM307 Investigation of a novel iron-uptake system in MecC Staphylococcus aureus

Claire Raisen[1]

[1]*Department of Veterinary Medicine, University of Cambridge, Cambridge, United Kingdom*

Background: *Staphylococcus aureus* (*S. aureus*) is a significant pathogen that causes a wide variety of disease in humans and animals. Methicillin resistant *S. aureus* (MRSA) isolates carrying *mecC,* the gene that confers resistance to the antibiotic, have been isolated from humans but also from diverse animal species covering livestock, domestic and wild animals throughout Europe. Many of the known MRSA *mecC* isolates have been whole-genome sequenced by our group to gain insight into the evolution and epidemiology of these emerging lineages.

Objectives: Here I describe the identification of a novel locus encoding a siderophore-like non-ribosomal peptide synthetase (NRPS), directly downstream of the *SCCmec* insertion site in *mecC S. aureus* isolates. A homologous region was identified in *Streptococcus equi* which encodes a NRPS that is involved in iron acquisition. I have therefore named the NRPS product 'staphylobactin' in MRSA, and the aim of this study was to determine the function of the staphylobactin biosynthesis cluster: is this region involved in iron acquisition and how might it be regulated?

Methods: In order to study the role of the staphylobactin gene cluster, deletion mutants of the staphylobactin locus were generated and tested in a number of phenotypic assays to identify a clear role for staphylobactin in iron metabolism.

Results: Although I was unable to generate definitive evidence revealing the biological role for the staphylobactin locus this study has generated valuable tools for further studies and thoroughly tested a number of hypotheses concerning its role in cation metabolism.

PM308 Resistant and pathogenic Staphylococcus aureus colonizing college coats

Isabela Rotta Batista[1], Amanda Caroline Lima Prates[1], Josimara Cristina Carvalho Araujo[1], Bruna Souza Santos[1], Yan Christian de Oliveira Bonfim[1], Glilciane Morceli[1], Jossimara Polletini[1], Marcus Vinícius Pimenta Rodrigues[1], Valeria Cataneli Pereira[1]

[1]*University of West Paulista, Microbiology, Presidente Prudente, Brazil*

Background: *Staphylococcus aureus* infections have presented considerable increase in healthy individuals and the colonization of biosafety equipment has contributed to the spread of this bacterium

Objectives: The study detected *S. aureus* from white coats of college students and to characterize this microorganism antimicrobial susceptibility and biofilm production.

Methods: Bacterial samples (n=300) were obtained from 100 college students' white coats in three regions: collar, sleeve and pocket. *S. aureus* were identified by Gram staining and catalase and coagulase tests. Antimicrobial resistance was tested by disk diffusion. The *mecA* gene and SCC*mec* was detected by PCR. The biofilm production was verified in Congo red (CRA) and the icaA and icaD genes were detected by PCR.

Results: *S.aureus* was identified in 45.0% of the samples. Antimicrobial resistance was observed in *S. aureus* samples for penicillin (72.59%), erythromycin (51.85%), cefoxitin (20.74%), oxacillin (17.04%), clindamycin (14.81%) and levofloxacin (5.18%) and the reduced susceptibility to vancomycin was detected in 9.6%. Methicillin resistant *S. aureus* was detected in 53.3% and SCC*mec* I, SCC*mec* III and SCC*mec* IV were detected in 52.8%, 25.0% and 11.1% of the samples, respectively. Biofilm production in CRA reached 94.0% and there was minimum concordance between CRA and *icaA* gene detection, while *icaD* gene did not presented any concordance with the CRA method. Most of the students (87.0%) had *S. aureus* colonized coats and biosafety measures need to be reinforced to avoid the spread of multiresistant and highly adhesive bacteria among other university sectors, hospitals and even relatives.

FAPESP (2016 / 122252-2 and 2016 / 15809-8)

PM309 Chimeric proteins to study the role of the transmembrane domain of the conjugative coupling protein TrwB

Itxaso Álvarez-Rodríguez[1], Carlos Garbisu[2], Itziar Alkorta[1]

[1]*University of the Basque Country, Biochemistry and Molecular Biology and Instituto Biofisika (UPV/EHU, CSIC), Leioa, Spain*
[2]*Neiker-Tecnalia, Department of Natural Resources Conservation, Derio, Spain*

Background: Infections caused by multidrug-resistant bacteria are one of the greatest threats to modern medicine and bacterial conjugation is the main mechanism for the spread of antibiotic resistance among bacteria. Coupling proteins (T4CPs) are essential elements of conjugative systems, and thus are ideal targets for inhibitors with putative application in the control of antibiotic resistant bacteria.

The coupling protein of conjugative plasmid R388, TrwB, is the paradigm of this family and is composed of a transmembrane domain and a cytosolic domain that contains the Walker A and Walker B motifs. It has been reported that the TMD plays a role in different features of the protein.

Objectives: To gain deeper knowledge in the role of the TMD of T4CPs, chimeric variants of TrwB have been constructed, switching its domains with other T4CPs and some characteristics of these chimeras have been compared to the properties of TrwB.

Methods: Matting assays were performed to determine whether the chimeric T4CP variants were active in plasmid transfer. Additionally, to study the location of these proteins confocal fluorescence microscopy was used.

Results: Our results show that the transmembrane domain plays a role in both the transfer capacity and in the subcellular location ofthese proteins. These results could be used in the future to find bacterial conjugation inhibitors and therefore to control antibiotic resistance spread among bacteria.

PM310 Prevalence of Ambler class A, B, and D beta-lactamases among clinical and nonclinical isolates of Pseudomonas aeruginosa in Serbia

Ina Gajic[1], Radoslava Savic Radovanovic[2], Bojana Lukovic[3], Natasa Opavski[4], Dusan Kekic[4], Vera Mijac[4], Snezana Jovanovic[5], Lazar Ranin[6]

[1]*Institute of Microbiology and Immunology*
[2]*Faculty of Veterinary Medicine, University of Belgrade*
[3]*College of professional health studies in Belgrade*
[4]*Medical Faculty, University of Belgrade*
[5]*Clinical Centre Of Serbia*
[6]*Medical Faculty, University of Belgrade*

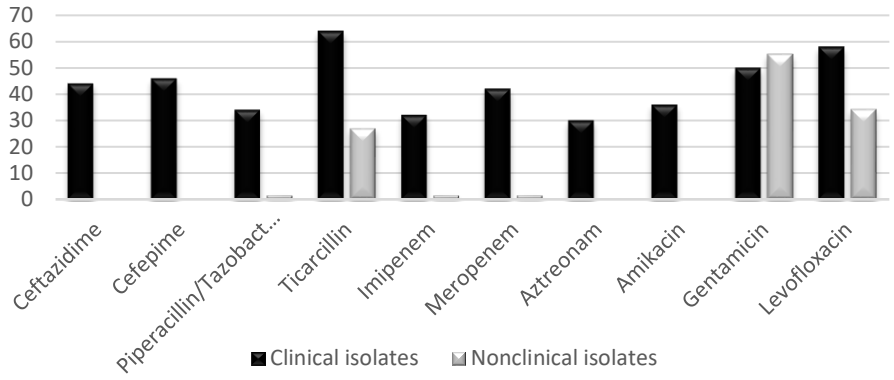
Background: Due to its intrinsic and acquired resistance to many common antimicrobial agents, *P. aeruginosa* has emerged as an important pathogen.

Objectives: The aims of this study were to evaluate the resistance rates of clinical and nonclinical isolates of *P. aeruginosa* and to determine the prevalence and genetic basis of Ambler class A, B and D β-lactamases.

Methods: A total of 117 clinical and nonclinical *P. aeruginosa* recovered from 2017-2018 were included in the study. The frequency of MBL (metallo-beta-lactamase) and ESBL (extended-spectrum beta-lactamase) producers was evaluated using combined disk diffusion tests. Genes encoding class A (bla_{PER}, bla_{VEB}, bla_{TEM}, bla_{SHV}, $bla_{CTX,}$ and bla_{GES}), class B (bla_{VIM}, $bla_{IMP,}$ bla_{NDM}) and class D β-lactamases (bla_{OXA-23}, bla_{OXA-24}, bla_{OXA-58}, and $bla_{OXA-143}$) were amplified by PCR.

Results: The antimicrobial resistance of the tested isolates are shown on Fig 1. The current study showed that clinical isolates were resistant to a higher number of antibiotics as compared with environmental isolates. A higher proportion of multidrug-resistant isolates was found among clinical isolates compared to nonclinical *P. aeruginosa* (30% vs. 9%; $p<0.05$). Six percent of isolates were confirmed as ESBL-producers (bla_{PER} gene-positive). The incidence of MBL-producing isolates among the carbapenem-resistant organisms was found to be 9.1%. MBL-positive isolates had an expression of the bla_{NDM} gene. None harboured other tested *bla* genes. Obtained results suggested that production of carbapenemases was not the major mechanism of carbapenem resistance among tested isolates.

Fig. 1. Antimicrobial resistance of the clinical and nonclinical isolates of *Pseudomonas aeruginosa*



PM311 Analysis of cytokine gene expression in initial infection of Mycobacterium avium subsp.paratuberculosis using epithelial cells-perperal blood mononuclear cells co-culture model

Hong-Tae Park[1], Hyun-Eui Park[1], Suji Kim[1], Soo Jin Shim[1], Woo Bin Park[1], Young Bin Im[1], Kuastros Mekonnen Belaynehe[1], Min-Kyoung Shin[2], Donghyuk Kim[3], Han Sang Yoo[1]

[1]College of Veterinary Medicine, Seoul National University, Department of Infectious Diseases, Seoul, Korea, Rep. of South
[2]Institute of Health Sciences, College of Medicine, Gyeongsang National University, Department of Microbiology, Jinju, Korea, Rep. of South
[3]Ulsan National Institute of Science and Technology, School of Energy and Chemical Engineering, Ulsan, Korea, Rep. of South

Background: Paratuberculosis is a chronic debilitating disease in cattle, which is caused by Mycobacterium avium subsp. paratuberculosis (MAP). Understanding host-MAP interactions in initial stage of infection is important to know how the MAP infected persistently in host evading their immune mechanisms. In this study, we designed epithelial cells-peripheral blood mononuclear cells (PBMCs) co-culture model to reveal host-pathogen interactomes.

Objectives: Establish a host-MAP interaction during initial stage of infection using an in vitro co-culture model.

Methods: Bovine MDBK cells and PBMCs were co-cultured by 3.0 µm pore size transwell. MDBK were seeded on apical chamber and PBMCs were seeded at lower chamber embedded with extracellular matrix made up with collagen I and human fibronectin. After 3 days incubation, MAP was infected with MOI 10:1 on apical chamber for 3 hours. Total RNA was extracted from the cells at 0h, 3h, 6h, 12h, 24h, 48h, 72h, 5d, 7d, and 10d pi and cytokine gene expression were compared by real-time qPCR.

Results: Several cytokine genes represent specific type of immune responses, Th1 and Th2 type immune response, were selected. Gene expression of IFN-γ was increased in infected PBMCs from 6h to 48h, and IL-12 was increased at 6h and 12h. However, IL-10 did not show any difference between two groups. The results suggested that Th1 type immune responses might be an initial response against MAP infection. This work was supported by the Strategic Initiative for Microbiomes in Agriculture and Food, MAFRA (No. IPET918020-4), the BK21 PLUS and RIVS, SNU, Korea.

PM312 Porcine Gastric Mucin Triggers Toxin Production of Enteropathogenic Bacillus cereus

Nadja Jessberger[1], Richard Dietrich[1], Ann-Katrin Mohr[1], Claudia Da Riol[1], Erwin Märtlbauer[1]

[1]*Department of Veterinary Sciences, Ludwig-Maximilians-Universität München, Oberschleißheim, Germany*

Background: Enteropathogenic *Bacillus cereus* cause food-borne infections due to the production of pore-forming enterotoxins in the intestine. Before that, spores have to be ingested, survive the stomach passage and germinate. Thus, before reaching epithelial cells, *B. cereus* comes in contact with the intestinal mucus layer.

Objectives: The impact of the mucus layer, especially mucins, on enteropathogenic *B. cereus* was studied.

Methods: RNA sequencing for total transcriptome analyses was performed, as well as mucin degradation, germination and motility assays. The enterotoxins were detected in *B. cereus* culture supernatants using enzyme immuno assays.

Results: Total RNA sequencing revealed major transcriptional changes of *B. cereus* strain F837/76 upon incubation with porcine gastric mucin (PGM), comprising genes encoding enterotoxins and further putative virulence factors, as well as proteins involved in adhesion to and degradation of mucin. Indeed, PGM was partially degraded by *B. cereus* via secreted, EDTA-sensitive proteases. The amount of enterotoxins detectable in culture media supplemented with PGM was also clearly increased. It was further shown that enterotoxin production upon contact with PGM is broadly distributed among *B. cereus* strains. Interestingly, evidence was found that PGM can also strain-specifically trigger germination of *B. cereus* spores and that vegetative cells actively move towards mucin.

Our data suggest that *B. cereus* is well-adapted to the host environment due to massive transcriptome changes upon contact with PGM, attributing mucin an important, so far neglected role in pathogenesis.

PM313 Quantitative and structural mass spectrometry analyses of host-pathogen interaction networks

Lotta Happonen[1], Hamed Khakzad[2], Simon Hauri[1], Lars Malmström[1;2], Johan Malmstrom[1]

[1]*Lund University, Department of Clinical Sciences Lund, Lund, Sweden*
[2]*University of Zurich, Institute of Computational Science and S3IT, Zurich, Switzerland*

Background: A fundamental challenge in medical microbiology is to quantitatively study protein-protein interaction networks or 'interactomes' arising at the interface between a pathogen and its host at a systems-wide level, with the potential of advancing the understanding of bacterial infections. Likewise, a central step towards the design of vaccines is to determine the quaternary structures and interaction interfaces of these host-pathogen protein-protein complexes.

Objectives: We apply data independent acquisition mass spectrometry (DIA-MS) and targeted crosslinking mass spectrometry (TX-MS) to describe the highly dynamic interactomes formed between the bacterium *Streptococcus pyogenes* and its host in order to determine how this pathogen evades the human immune defense.

Methods: Using a combined affinity-purification and surface-adsorption DIA-MS approach, we search for *S. pyogenes* - human interactions arising in plasma and saliva mimicking invasive infections and infections in the upper respiratory tract. We further describe a high-resolution quaternary human interactome model of the major *S. pyogenes* surface antigen the M protein using TX-MS.

Results: The complementary affinity-purification and surface-adsorption strategies identify over 200 interactions between *S. pyogenes* and its host and describes how the host-pathogen interaction networks are regulated. We demonstrate that secreted streptococcal proteins tend to interact with human saliva proteins whereas cell wall-attached proteins interact with human plasma proteins, implicating that *S. pyogenes* has developed distinct strategies for evading the host defense in different environments. Furthermore, our data suggest vulnerable M protein epitopes of importance for *S. pyogenes* interaction and internalization during phagocytosis, with possible applications for the future development of treatment strategies.

PM314 Epigenetic reprogramming of human macrophages by pathogenic Yersinia

Indra Bekere[1], Marie Schnapp[1], Laura Berneking[1], Jiabin Huang[1], Thomas Günther[2], Martin Aepfelbacher[1]

[1]*University Medical Center Hamburg-Eppendorf (UKE), Institute of Medical Microbiology, Virology and Hygiene, Hamburg, Germany*
[2]*Heinrich Pette Institute, Leibniz Institute for Experimental Virology, Hamburg, Germany*

Background: The pathogenic *Yersinia pestis*, *Y. pseudotuberculosis* and *Y. enterocolitica* suppress the host immune response by downregulating the transcription of inflammatory genes. This activity is encoded on a virulence plasmid which allows the bacteria to inject effector proteins (Yops) into the host cells via the bacterial type three secretion system.

Objectives: We investigated whether epigenetic histone modifications play a role in the global effect of *Y. enterocolitica* on inflammatory gene transcription.

Methods: Therefore we infected primary human macrophages with *Y. enterocolitica* strains (wild type, ΔYopM and ΔYopP mutants and the virulence plasmid deficient WAC strain) and performed Chromatin Immuno-Precipitation (ChIP)-seq employing anti-H3K4me3, -H3K27ac, -H3K27me3 and -H3K4me1 antibodies in combination with RNA-seq.

Results: Wild type induced activation of ~3000 promoters, which dose dependently paralleled transcription of the associated genes. Further, wild type and avirulent *Yersinia* strongly upregulated activating H3K27ac at enhancers of more than 6000 and 3000 genes, respectively. Integration of all data revealed that wild type but not WAC upregulated promoters and enhancers of genes for chromatin organisation and metabolism. In addition, wild type suppressed activation of promoters and enhancers of immune response genes, many of which were upregulated by WAC. YopM did not to play a significant role while YopP showed a small contribution to the wild type effects, suggesting that Yops other than YopP or YopM or the interplay of all Yops contribute to this phenomenon. In summary, we provide evidence for a profound epigenetic reprogramming of macrophage metabolism and inflammatory response by virulent *Y. enterocolitica*.

PM315 Quorum sensing phenotypes of bdsf signaling mutant in burkholderia contaminans sk875

Hae-In Jung[1], Yun-Jung Kim[1], Soo-Ki Kim[1]

[1]*Konkuk University, Department of Animal Sciences and Technology, Seoul, Korea, Rep. of South*

Background: *Burkholderia contaminans* SK875 is a gram-negative bacteria isolated from the respiratory tract of a pig. The *rpfF* gene catalyzes the production *cis*-2-dodecenoic acid (BDSF) which interacts with the receptor *rpfR* encoding the cyclic di-GMP phosphodiesterase gene comprised of PAS-GGDEL-EAL domains. N-Acyl homoserine lactone and BDSF quorum sensing (QS) systems regulate bacterial virulence but their relation is still not completely understood.

Objectives: *B. contaminans* SK875 (*rpfR*::Tn) insertion mutant was tested for the QS phenotypes. To find molecular networks in BDSF signaling, we have characterized a set of deletion mutants for the *rpfF* and *rpfR* genes.

Methods: The transposon (Tn5) insertion mutant of SK875 with longer life span of *Caenorhabditis elegans* was screened in the library. An allelic exchange system was used to construct BDSF signal deletion mutants and then QS phenotypes were determined.

Results: The Tn5 was positioned to the cyclic di-GMP phosphodiesterase gene. TLC analysis of insertion mutant displayed decreased autoinducer secretion, which correlated well with the results of plate bioassay and luminescence. The scanning electron microscopy of the insertion mutant showed a fibril structure composed of overproduced biofilm. Furthermore, the deletion mutants of *rpfF*, *rpfR*, and *rpfFR* displayed increased biofilm production but with reduced swimming and swarming motilities. A longer life of *Galleria mellonella* was observed after feeding with the deletion mutants as compared with the wild type. These results suggested that the BDSF signal might play a mediate role on QS-based virulence by inhibiting biofilm formation and promoting bacterial motility.

PM316 Differential Gene Expression of Burkholderia cenocepacia BCAM2418 Trimeric Autotransporter Adhesin in the initial steps of bacteria-host interaction

Andreia Pimenta[1;2], Dalila Mil-Homens[1], Arsénio M. Fialho[1;2]

[1]*Institute for Bioengineering and Biosciences (iBB), Instituto Superior Técnico, University of Lisbon, Lisbon, Portugal*
[2]*Department of Bioengineering, Instituto Superior Técnico, University of Lisbon, Lisbon, Portugal*

Background: Bacterial initial contact to host cells is a crucial step in host-pathogen interaction. Bacterial adherence to the host could lead to a prompt transcription of virulence-associated genes. *Burkholderia cenocepacia* is a human pathogen known for its adherence and interaction with the host, causing severe lung infection in cystic fibrosis (CF) patients. The machinery used to promote host contact is wide and complex. Trimeric Autotransporter Adhesins (TAAs), a class of extracellular proteins, are key elements in early steps of *B. cenocepacia* infection, facilitating adherence to lung cells. *B. cenocepacia* encodes for 7 TAAs known to be multifunctional proteins involved in many virulence traits.

Objectives: Uncover the relevance of one *B. cenocepacia* TAA in the early-stages of infection.

Methods: Expression levels of *B. cenocepacia* TAA genes were screened after adhesion to bronchial cells. *Bcam2418* TAA gene reached transcription values higher than all other tested TAAs. Additionally, its expression was evaluated in a set of CF-related conditions, after adhesion to four epithelial cell lines and to mucin-coating. Bronchial cells were subject to proteolytic treatments and *bcam2418* expression analyzed after adhesion.

Results: From the studied CF-related conditions, adhesion to bronchial epithelium and to mucins prompted the higher increase in *bcam2418* expression, which seems to follow a time-line pattern. *Bcam2418* transcription after bacterial adhesion to treated bronchial epithelium are lower when compared to an untreated cellular monolayer. Results showed that expression of *bcam2418* vary over time and seems to be specifically triggered by pathogen-cell interaction through a protein-like membrane component recognized by the bacteria.

PM317 Adherence to human epithelial cells and biofilm formation by multidryg resistance Citrobacter strains isolated from solid organ transplant recipients (The ENTHERE study)

Itziar Chapartegui-González[1], Marta Fernández-Martínez[1;2;3], Claudia González-Rico [1;2;3], John Barret[1], Félix Acosta[4], María Carmen Fariñas[1;2;3], Jose Ramos Vivas[1;2]

[1]IDIVAL Research Institute, Microbiology
[2]REIPI (Spanish Network for Research in Infectious Diseases)
[3]University Hospital Marqués de Valdecilla
[4]University of Las Palmas de Gran Canaria

Background: *Citrobacter* represents an understudied member of the Enterobacteriaceae family with relevance to healthcare-associated opportunistic infections. Recent findings on antimicrobial resistance in Citrobacter calls for further efforts to examine the significance of *Citrobacter* in the clinical settings. Furthermore, the host cell response to these bacteria is not well understood.

Objectives: Our aim was to analyse the capacity to adhere to, and to form biofilms of MDR Citrobacter strains isolated pre- or post-transplant patients.

Methods: The adherence of 13 MDR *Citrobacter* strains, isolated from rectal swabs before or after kidney or liver transplant patients from the H.U.M.Valdecilla, to the human colon cell line HT-29 was evaluated by using immunofluorescence microscopy. The biofilm-forming capability of these strains was also evaluated by using a microplate assay and confocal microscopy.

Results: We found 5 strains (38%) with diffusely adherent phenotype and 2 strains (15%) with high aggregative adherence to HT-29 human colon cells. The adherence in these strains was not inhibited by mannose. Using a polyclonal antibody, we showed that although the morphology of bacteria was similar, the pattern of fluorescence changed in several strains indicating a very different antigenicity.

Our study shows that biofilm formation by the MDR *Citrobacter* strains increases during growth at 25ºC but not at 37ºC, however, the correlation between adhesion to human cells and biofilm formation was strain dependent. This investigation could contribute to a better understanding of the relationship between adherence capability, antimicrobial resistance and pathogenicity of MDR bacteria in solid organ transplant recipients.

PM318 Evaluation of treatment coverage of the Colombia tuberculosis free strategic plan 2006-2015

Wellman Ribon[1;2], Lina Maria Vera[1], Juan Pablo Gutierrez[2], María Cecilia Gonzalez[2], Carolina Becerra[3]

[1]*Universidad Industrial de Santander*
[2]*Instituto Nacional de Salud Pública de México*
[3]*Secretaría de Salud de Bucaramanga*

Background: The obligatory compliance with the strategy "DOTS" was defined since 2000. As part of the 2006-2015 Tuberculosis Free Colombia Strategic Plan (PECLT), the goal was to achieve 85% coverage. The behavior of this indicator has been evaluated, so the impact of the Plan is unknown.

Objectives: Evaluate the behavior of the treatment, as an indicator of the Strategic Plan Colombia Free de Tuberculosis, 2006-2015.

Methods: An interrupted time series approach was used to evaluate level and trend changes in treatment coverage. 2006 was considered as the potential turning point, corresponding to the implementation of the Plan in Colombia.

Results: The success in the treatment, reported by the World Bank, presented a downward trend in 0.3 units, reached 4.9% in 2006, and thereafter it increased 0.8 units annually. When comparing the Plan with the non-implementation, an increase, not significant, of 0.5% was obtained annually. According to Ministry notifications, this indicator was increasing ($p < 0.001$); however, in 2006 it decreased 3.2%, and annually thereafter, this decrease was significant in 10.6%. In general, the implementation of the Plan contributed to the non-significant increase of 0.07 percentage units. Conclusions: It is necessary to evaluate, in addition to the treatment, all the indicators that make up the PECLT, from mixed methodologies, in order to identify the impact of the Plan, taking into account all the potential variables that could have influenced the outcomes.

PM319 Human gut symbionts suppress toxin release from Clostridioides difficle

Tomomi Kuwahara[1], Miad Elahi[1], Haruyuki Nakayama-Imaohji[1], Ayano Tada[1]

[1]*Kagawa University, Microbiology, Kagawa, Japan*

Background: Adult human gut microbiota is typically dominated by two bacterial phyla, Firmicutes and Bacteroidetes. Since the Firmicutes includes toxin-producing species such as *Clostridioides difficile* (CD) and *Clostridium perfringens*, molecular basis of the interaction among these dominant phyla is an important focus to elucidate the role of the gut symbionts on human health. CD-associated diarrhea (CDAD) is induced by the disruption of normal gut microbiota by antibiotics. Amount of the toxins produced by CD in the gut is a critical factor for the development of CDAD. It is still unknown whether the human gut microbiota modulates the toxin synthesis of the enteric pathogen.

Objectives: In this study, we determined whether human gut symbionts, *Bacteroides*, inhibit the toxin synthesis of CD or not.

Methods: CD was grown in the culture supernatants of selected human gut symbionts, and CD toxin levels were quantified. Furthermore, the transposon-based mutagenesis was performed on selected gut microbes to identify the genes responsible for the anti-toxinogenicity against CD.

Results: We found that the culture supernatant of *Bacteroides thetaiotaomicron* (BT) prevents the toxin synthesis of CD. The biochemical analysis suggested that the candidate molecules in BT culture supernatant are heat resistance and range from 10 to 100 kDa in size. Screening of 2,500 transposon-inserted BT mutants identified several clones that reduced the anti-toxinogenicity against CD. In these mutants, transposon inserted in the genes associated with carbohydrate metabolism and polysaccharide biosynthesis. The results suggest that the polysaccharides produced by BT modify the toxin production of CD.

PM320 Mycobacterium tuberculosis inhibits autocrine type I interferon signaling to increase intracellular survival

Dallas Banks[1], Sarah Ahlbrand[1], Keith Hughitt[1], Swati Shah[1], Stefanie Vogel[2], Najib El-Sayed[1], Volker Briken[1]

[1]*University of Maryland, Cell Biology and Molecular Genetics, College Park, United States*
[2]*University of Maryland School of Medicine, Microbiology and Immunology, Baltimore, United States*

Background: The type I interferons (IFN-α and -β) are important for host defense against viral infections. In contrast, their role in defense against non-viral pathogens is more ambiguous.

Objectives: We analyzed the role of IFN-β-signaling on intracellular survival of *Mycobacterium tuberculosis* (Mtb) and the impact of Mtb infection on the IFN-β signaling pathway.

Methods: Primary murine macrophages were infected with Mtb and cell signaling was analyzed by Western blotting and transcriptome analysis via RNAseq.

Results: Here we report that IFN-β-signaling in macrophages has protective capacity against *Mycobacterium tuberculosis* (Mtb) via the increased production of nitric oxide. Furthermore, Mtb is able to inhibit IFN-α/β-receptor-mediated cell signaling and the transcription of 309 IFN-β stimulated genes which includes genes associated with innate host cell defense. The molecular mechanism of inhibition by Mtb involves reduced phosphorylation of the IFNAR-associated protein kinases JAK1 and TYK2 leading to reduced phosphorylation of the downstream targets STAT1 and STAT2. Overall, our study supports the novel concept that Mtb evolved to inhibit autocrine type I IFN signaling in order to evade host defense mechanisms.

PM321 Impact of prolonged incubation of susceptibility plates on antibiotic disks zones

Dane Luznik[1], Viktorija Tomic[1], Vesna Spendal[1], Marusa Ravnik[1]

[1]*University Clinic of Respiratory and Allergic Diseases Golnik, Laboratory for Respiratory Microbiology, Golnik, Slovenia*

Background: Disk diffusion is one of the most widely used antimicrobial susceptibility testing (AST) methods in routine clinical microbiology laboratories. The method is robust and is suitable for testing the majority of bacterial pathogens.

Objectives: One of the parameters of standardised disk diffusion AST is length of incubation of inoculated plates. We wanted to determine impact of prolonged incubation of susceptibility plates on antibiotic disks zone sizes and interpretation of results.

Methods: 28 different bacterial isolates (6 different bacterial families) were collected in august 2018 in University Clinic of Respiratory and Allergic Diseases Golnik. Antibiotic disks used in routine susceptibility testing (13 different antibiotic groups) were tested. Zones of inhibition around antibiotic disks were measured after 18-hours and 48-hours incubation. After each incubation period, 283 zone measurements were performed. Measurements after 48-hours incubation were compared to measurements after standard 18-hours incubation and the differences between two measurements (in %) were calculated.

Results: Detected differences were either an increase or decrease in zone size. Standard deviation was calculated from all measurements. Result 2σ was 5,33%, which means that 95,45% of all measurements after 48h incubation deviated less than 5,33% from measurements after 18h incubation. Trend to a universal increase or decrease of zone readings after 48 hours compared to 18 hours incubation could not be determined. Measurement deviations were random and are very likely a consequence of measurement inaccuracy. Since none of the measurement deviations changed susceptibility interpretation, we concluded that 48 hour incubation can be used in certain situations.

PM322 Nasal colonization by methicillin-resistant Staphylococcus aureus in professionals of the Brazilian program "Family Heath Strategy"

Letícia Calixto Romero[1], Isabella Carolina Rodrigues dos Santos Goes[1], Ana Julia Turra[1], Talyssa Fernandes Silva de Oliveira Rodrigues[1], Maria Angelica Gotardi[1], Glilciane Morceli[1], Lizziane Kretli Winkelstroter Eller[1], Valeria Cataneli Pereira[1]

[1]*University of West Paulista, Microbiology, Presidente Prudente,*

Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the major microorganisms involved in infections at health facilities and at community level. A significant proportion of these infections appear to result from cross-contamination between the patient and the health-care worker. Health professionals are at the interface between hospitals, long-term care facilities and nursing homes, on the one hand, and the community, on the other, can serve as reservoirs, vectors or victims of cross-transmission of MRSA

Objectives: The objective of this study was to determine the prevalence of MRSA from the nasal cavity of professionals working of a health and verify the Biofilm-formation ability.

Methods: Nasal samples were collected from 63 employees from the program defined as Family Health Strategy (FHS), from the city of Pirapozinho / SP, Brazil. *S. aureus* was identified by PCR. The *mecA* gene was performed by PCR and the MRSA were classified according to the Staphylococcal Chromosome Cassette *mec* (SCC*mec*) by multiplex PCR

Results: Of the 63 employees, 74.6% were positive for *S. aureus*. Of these, 69.5% were MRSA with SCC*mec*I (24.4%), SCC*mec* III (34.1%) and SCC*mec* III (36.6%). In addition, all strains showed biofilm production. The results confirm the high occurrence of MRSA among FHS employees, which shows us the need to resort to the dissemination control techniques of these multiresistant strains, broadening the perspective of prevention of infections related to health care, covering all levels of currently under-exploited, such as basic health units.

FAPESP (2017 / 01104-5)

PM323 AmpC-dependent resistance of Pseudomonas aeruginosa to Ceftolozane/Tazobactam

Melanie Grosjean[1;2;3], Cédric Muller[1], Patrick Plésiat[2;3]

[1]*SMALTIS SAS, Besançon, France*
[2]*UMR 6249 ChronoEnvironnement, Besançon, France*
[3]*CNR de la Résistance aux Antibiotiques, CHRU Jean Minjoz, Besançon, France*

Background: Resistance of clinical strains of *Pseudomonas aeruginosa* to beta-lactams can arise from mutations associated with changes in intrinsic beta-lactamase AmpC. We previously showed that several single point mutations in AmpC increase the hydrolytic activity of the enzyme towards ceftolozane/tazobactam (C/T) and thus compromising the clinical efficacy of this treatment.

Objectives: Here, we explore the effects on C/T Minimal Inhibitory Concentrations of concomitant mutations in AmpC to anticipate a possible evolution of clinical strains in response to an increasing use of C/T.

Methods: 28 new amino acid variations in AmpC were identified among a collection of beta-lactam resistant strains of *P. aeruginosa* collected in France. These amino acid changes were individually or collectively introduced by site-directed mutagenesis in the AmpC coding gene (bla_{AmpC}) from reference strain PAO1. Resistance profile of strain PAO1Δ$bla_{AmpC:PAO1}$ overproducing these AmpC variants was determined by standard protocols.

Results: 10 single point variations turned out to increase the resistance of PAO1Δ$bla_{AmpC:PAO1}$ to C/T from 2- to 32-fold as compared with wild-type AmpC. A series of AmpC variants containing 2, 3, 4 or 5 novel or previously characterized mutations were constructed. The studied mutations had different and non-predictable impacts on C/T MICs with some combinations exhibiting antagonistic, neutral or synergistic effects. This work shows that multiple amino acid variations can increase the hydrolytic activity of AmpC on C/T and other cephalosporins. Lines of evidence suggest that accumulation of such mutations by AmpC overproducing *P. aeruginosa* can be an evolutionary trajectory to gain a very high resistance to C/T.

PM323 Identification of target recognition domains in EspL from enteropathogenic E. coli

Anna Katharina Riebisch[1], Petra Dersch[2], Sabrina Muehlen[2]

[1]*Helmholtz Centre for Infection Research, Systems-Oriented Immunology and Inflammation Research, Braunschweig, Germany*
[2]*Helmholtz Centre for Infection Research, Molecular Infection Biology, Braunschweig, Germany*

Background: Enteropathogenic *Escherichia coli* (EPEC) are non-invasive gastrointestinal pathogens that translocate bacterial effector proteins into the host cell. These effectors are able to subvert host cell signaling in favour of bacterial survival and replication and can modulate the host immune response to infection. Recently, we have identified and characterized the role of a number of EPEC effector proteins that block the production and release of pro-inflammatory cytokines as well as apoptotic cell death. More recently, we identified the effector protein EspL as a novel cysteine protease that specifically cleaves the receptor-interacting protein kinases (RIPKs) 1 and 3 in their RHIM domain, resulting in the abrogation of the caspase-independent form of cell death, necroptosis.

Objectives: Here, we aim to identify the area(s) of the EspL protein responsible for target recognition and cleavage.

Methods: We used co-transfection and immunoblotting to evaluate RIPK cleavage.

Results: We found that the C-terminal 150 amino acids (EspL399-459) are completely dispensable for EspL-mediated cleavage of RIP kinases. Using random transposon-based insertion mutagenesis, we identified 5 insertion sites, which affected cleavage of RIPK3. Interestingly, site-directed mutagenesis of the amino acids surrounding the insertion sites had no effect on target cleavage. However, closer analysis predicted that the transposon insertions disrupted the ankyrin repeat structure of the C-terminal domain of EspL, hence interfering with the number of or distance between ankyrin repeats. This suggests that the sequence of the ankyrin repeats interacting with the target may be negligible. Future studies are required to test this hypothesis.

PM324 Differentiating Entameoba hystolytica, E.dispar and E. moshkovskii in stool sample from rural community of Nepal using Nested-PCR

Sandeep Thapa[1], Ajaya Kunwar[1], Nilam Thakur[1], Govardhan Joshi[1], Rajesh Das[2]

[1]*Kathmandu Center for Genomics and Research Laboratory, Microbial Genetics, Gwarko, Nepal*
[2]*Kathmandu College of Science and Technology, Microbiology, Kathmandu, Nepal*

Background: Nepal is a developing country with numerous health complications. Amebiasis, an infectious disease caused by *Entamoeba* species is extremely observed in rural area of Nepal.Current accounts affirmed that open defecation, drinking untreated water, unhygienic habits and inadequate knowledge of basic health are the prominent causes of higher mortality and morbidity in our country.Likewise, *E. histolytica, E. dispar,* and *E. moshkovskii* are morphologically identical but are biochemically and genetically different.

Objectives: The aim of this study is to ddetect and differentiate *Entamoeba* species in the diarrhoeal patient.

Methods: A total of 270 faecal samples were collected from south eastern terai region of Nepal after the informed consent form and processed by direct wet smear and formalin ethyl acetate concentration technique. Microscopic examination was performed for the detection of *Entamoeba* species along with other intestinal parasites. Additionally, Nested- PCR targeting 16S-like ribosomal RNA gene was carried out for characterisation of microscopically positive faecal samples containing the E complex.

Results: 67.3% were male whereas only 32.7% were female among collected samples. Microscopy-positive faecal samples containing the E complex (n = 56) were subject to PCR. Surprisingly, out of PCR-positive samples (n=46), only three samples were reported to contain *E. histolytica*. Likewise, viral diarrheal was most significant form of diarrhoea found in 76.67% of patients. Among different organisms, *As. Lumbricoids* and *E. histolytica, G. lambia* and *H. Nana* were identified in most of the patients accounting for 11.11%, 8.52%, 2.59% and 1.11% respectively.

PM325 Role of genetic and epigenetic modifications in the bacterial plant pathogen Ralstonia solanacearum during adaptation to the resistant tomato Hawaii 7996 plant

Rekha Gopalan Nair[1;2], Ludovic Legrand[1;3], Xavier Barlet[1;3], Stephane Genin[1;3], Alice Guidot[1;2;3]

[1]INRA Institut National de Recherche Agronomique-Laboratoires, Laboratoire des Interactions Plantes-Microorganismes, Auzeville-Tolosane, France
[2]Université Toulouse Paul Sabatier, Toulouse, France
[3]C.N.R.S., Toulouse, France

Background: *Ralstonia solanacearum,* the causal agent of bacterial wilt disease infects more than 250 plant species. The ability of this pathogen to adapt to many host plants and counteract plant resistance is supported by field observations reporting strains more aggressive and able to colonize novel hosts. To investigate the genetic/epigenetic bases of host adaptation in *R. solanacearum,* experimental evolution was conducted with GMI1000 strain on tomato Hawaii 7996 plant, a reference cultivar resistant to bacterial wilt, by serial passage experiments (SPE) during 300 bacterial generations. The experimentally evolved clones are better adapted to tomato Hawaii. ***Hypotheses:*** Both genetic and epigenetic modifications could explain fitness gain of these clones.

Objectives: The global objective is to investigate the role of genetic/epigenetic (DNA methylation) modifications in *R. solanacearum* during experimental adaptation to resistant tomato Hawaii plant.

For that purpose, fitness gain of the experimentally evolved clones will be measured in tomato Hawaii; genome, methylome and transcriptome of these clones will be compared to the ancestral GMI1000; and modifications in the genomic/methylome profile will be characterized.

Methods: Competitive index (CI) experiment was conducted to analyze fitness gain *in planta.* Genomic sequence analysis was performed by Illumina sequencing; methylome analysis by PacBio SMRT sequencing and bisulfite treatment/Illumina sequencing; and RNA-seq by transcriptome analysis.

Results: The 25 tested evolved clones had increased *in planta* fitness compared to the ancestral clone. Whole genome sequencing of evolved clones showed between 0 and 1 polymorphism per clone. Methylome and transcriptome analyses of these clones are currently being run.

PM326 Interference with actin-based bacterial dissemination by the human host defense protein hGBP1

Miriam Kutsch[1], Jorn Coers[2]

[1]Duke University, Molecular Genetics and Microbiology, Durham,
[2]Duke University, Molecular Genetics and Microbiology, Durham, United States

Background: Several species of invasive bacteria including the human-adapted, enteric pathogen *Shigella* enter host cells via endocytosis and subsequently escape from an intracellular vacuole into the host cell cytosol. Survival within the cytosolic milieu drove the evolution of specific niche adaptations. One adaptation commonly found amongst intracytosolic bacterial pathogens such as *Shigella* is the ability to hijack the host's actin polymerization machinery in order to generate force for intracytosolic movement and the invasion of neighboring host cells. This actin-based motility enables *Shigella* to spread within the colonic epithelium while simultaneously avoiding extracellular immune mechanisms. Host defense against *Shigella* is therefore critically dependent on cell-autonomous defense programs. One such cell-autonomous defense is executed by the dynamin-related GTPase human guanylate-binding protein 1 (hGBP1). We previously reported that hGBP1 binds to *Shigella* inside the host cell cytosol and blocks bacteria from utilizing the host actin polymerization machinery for intracytosolic motility and cell-to-cell spread.

Objectives: The mechanisms underlying this potent hGBP1-mediated defense program is currently unknown and represents a critical gap in knowledge. The goal of this study is to define the molecular mechanisms by which hGBP1 prevents actin-based motility of *Shigella.*

Methods: We apply cell biological, biochemical and genetics approaches to define the antimicrobial function of hGBP1.

Results: We demonstrate that hGBP1 blocks the recruitment of actin nucleation factors to the bacterial pole and provide further insights into the underlying molecular mechanism.

PM327 Comparative genomics of mycoplasmas from North Atlantic seals

Daniel Brown[1], Dina Michaels[1], Jessy Castellanos Gell[2], Gerald Kutish[3], Salvatore Frasca Jr.[2]

[1]*University of Florida, Infectious Diseases and Immunology, Gainesville, Florida, United States*
[2]*University of Florida, Comparative, Diagnostic, and Population Medicine, Gainesville, Florida, United States*
[3]*University of Connecticut, Pathobiology and Veterinary Science, Storrs, Connecticut, United States*

Background: Episodic seal die-offs documented along northern coasts of the Atlantic Ocean from 1980 to the present have been attributed to morbillivirus or influenzavirus outbreaks involving pneumonia with systemic disease. Species of *Mycoplasma* were also detected in tissues of moribund or dead seals.

Objectives: We conducted comparative genome analyses to search for genetic features referable to host range, tissue tropisms, or virulence of the mycoplasmas most frequently associated with those cases.

Methods: The genomes of *Mycoplasma phocicerebrale* 1049^T and *Mycoplasma phocirhinis* 852^T were sequenced using the Illumina MiSeq system, assembled and annotated using RASTtk and PGAP pipelines as we previously described for *Mycoplasma phocidae* 105^T. The data were deposited into GenBank as Bioprojects PRJNA473817 and PRJNA505803 respectively.

Results: The 865 kb closed genome of *M. phocirhinis* encodes 676 proteins, versus the 608 proteins in the 814 kb genome of *M. phocidae*. The differences are in part because *M. phocirhinis*, the only species lacking a CRISPR system, is riddled with IS3 and IS1634 family transposases. The 743 kb linear assembly achieved for *M. phocicerebrale* encodes 613 proteins. The genes having assigned roles partitioned into functional subsystems similarly across species. All species encode orthologs of mycoplasmal protein M, which binds host IgG to block antigen-specific antibody binding. No other determinants of tissue tropism or virulence were discerned in the genomes of *M. phocicerebrale* or *M. phocirhinis*. It remains to be confirmed whether immune modulation by mycoplasmosis predisposes susceptibility or influences outcomes of intercurrent diseases of seals.

PM328 Exploration on diarrheal pathogenicity induced by patient-derived proteus mirabilis infection in mice

Zelong Gong[1], Fang Bai[2], Yubin Li[1], Min Long[1], Xiaolu Shi[2], Hong Cao[1]

[1]Department of Microbiology, School of Public Health, Southern Medical University
[2]Shenzhen Major Infectious Disease Control Key Laboratory, Shenzhen Center for Disease Control and Prevention

Background: *Proteus mirabilis* is commonly classified as opportunistic pathogen, and may induces urine tract infection (UTI) among human. Accumulating evidences indicate that some novel strains of *P. mirabilis* were responsible for sporadic outbreaks of food poisoning in China. However, its pathogenic mechanism remains unclear.

Objectives: To explore the diarrheal pathogenicity of patient-derived *proteus mirabilis in vivo*.

Methods: Strains of *P. mirabilis* involved in the experiment were isolated from different specimens (C02011 isolated from patient's vomit/feces, B02005 isolated from health people's feces). The experiments were performed in 6 weeks' BALB/c male mice to explore the pathogenicity of isolated strains. 36 mice randomly divided into three groups (C02011/B02005/Control) were orally administrated *P. mirabilis* C02011 (2.5×10^8 CFU), B02005 (2.5×10^8 CFU) and saline water, respectively. After infection, mice physical status (body weight), feces status (fecal characters, fecal water content) and anatomic status (colon length, histological sections) were monitored and detected in real time.

Results: In the results, the body weight of C02011 mice decreased within 3 h post-challenge, comparing with B02005 and control mice. Loose feces were also observed in C02011 group after infection, along with the increase of fecal water content ($P<0.05$). Additionally, inflammation symptoms were provoked in the large intestine of C02011 mice, in accordance with the shortening colon lengths ($P<0.05$) and representative histopathological examination. These results demonstrated that a diarrheic mice model induced by patient-derived *P. mirabilis* was applied to explore the diarrheal pathogenicity of different strains preliminarily. (*Corresponding author: Hong Cao, E-mail: gzhcao@smu.edu.cn)

PM329 Antimicrobial activity of three natural plant extracts of Zataria multiflora, Myrtus communis and Peganum harmala on Extended spectrum beta-lactamases-producing P. aeruginosa strain

Saeed Shams[1], Ali Hashemi[2], Mohammad Barati[3]

[1]*Qom University of Medical Sciences, Cellular and Molecular Research Center, Qom, Iran*
[2]*Shahid Beheshti University of Medical Sciences, Department of Microbiology, School of Medicine, Tehran, Iran*
[3]*AJA University of Medical Sciences, Infectious Diseases Research Center, Tehran, Iran*

Background: Extended spectrum-beta Lactamases (ESBLs) and Metallo-beta Lactamase (MBL)-producing bacteria are a worldwide health concern. *Pseudomonas aeruginosa* is one opportunistic bacterium, causing a wide variety of infections, especially in burns.

Objectives: Our aim was to detect ESBL and MBL-producing *P. aeruginosa* isolated from burned patients and to evaluate antibacterial effects of the methanol extracts of *Zataria multiflora, Myrtus communis*, and *Peganum harmala* on the resistant strains.

Methods: Sampling was done from 245 burn wounds of the hospitalized patients. After identifying bacteria by biochemical methods, MIC was carried out for cefotaxime, meropenem, aztreonam, imipenem, and ceftazidime according to CLSI guideline. ESBLs and MBLs-producing isolates were also detected using double disk synergy test and E-test strips, respectively. Methanol extracts of the plants were prepared by percolation method and MIC (in concentrations of 1.6, 0.8, 0.4, 0.2, 0.1, 0.05, and 0.025 mg/ml) was evaluated by microdilution assay. *P. aeruginosa* ATCC 27853 was used as a control strain.

Results: Out of 245, 120 patients were infected with *P. aeruginosa*. The most resistance was detected for ceftazidime (66%). ESBL was identified in 41 isolates (16.73%), while MBL could not be identified among strains. *Zataria multiflora* showed the highest antibacterial effect on the strains in concentration of 0.025 mg/ml. Our results showed that ESBL-producing *P.aeruginosa* strains are increasing in our geographical area. Regarding to relatively high levels of the antibiotic resistance, the use of herbal medicinal products such as *Zataria multiflora* may be considered as an appropriate agent for treatment; however, more investigations are needed.

PM330 Whole genome MLST provides ample resolution for typing and outbreak detection of Cronobacter spp.

Dieter De Coninck[1], Lysiane Hauben[1], Margo Diricks[1]

[1]*BioMérieux, Data Analytics Unit, Sint-Martens-Latem, Belgium*

Background: Cronobacter spp. causes infections such as meningitis, septicaemia and necrotizing enterocolitis in neonates and immunocompromised adults. Although infections are rare, they are often serious for young infants, leading to death.

Objectives: Following several fatal outbreaks in neonatal intensive care units (NICU), the World Health Organization in 2004 requested the establishment of a molecular typing method to enable international control of the organism. Nowadays, whole genome sequencing can be performed for the equivalent cost of classical MLST typing.

Methods: We developed a whole genome MLST (wgMLST) scheme for Cronobacter by expanding the cogMLST scheme developed by Forsythe et al. (2014) to the pan-genome. By also capturing the accessory loci (13,862), the discriminatory power increased.

Results: The quality of the scheme was assessed by comparing its performance to whole genome SNP (wgSNP). This resulted in the same phylogeny. The wgMLST approach was easier scalable and computationally less intensive. We applied the scheme to one of the largest described outbreaks in a NICU in France (1994) that lasted over 3 months and claimed the lives of three neonates. wgMLST analysis of the 26 outbreak isolates led to the same conclusions as the original study.

The wgMLST scheme for Cronobacter spp. provides ample resolution for typing and detection of outbreaks. It provides better resolution compared to classical MLST and cogMLST. The possibility to extract traditional typing data, resistance and virulence data from the wgMLST scheme reduces the total analysis time and cost, and may lead to more efficient outbreak detection.

PM331 Staphylococcus aureus Pathogenicity Islands control chromosomal virulence genes expression

Mercedes Cervera-Alamar[1], Katerina Guzmán-Markevitch[2], Alejandro Toledo-Arana[3], Iñigo Lasa[4], Jose Penades[5], Mª Ángeles Tormo-Mas[2]

[1]Health Research Institute, Severe Infection Group, Valencia, Spain
[1]Health Research Institute, Severe Infection Group, Valencia, Spain
[3]Instituto de Agrobiotecnologia, Bacterial Gene Regulation group, Navarra, Spain
[4]Navarrabiomed, Laboratory of Microbial Pathogenesis, Pamplona, Spain
[5]University of Glasgow, Institute of infection, immunity & inflammation, Glasgow, United Kingdom

Background: *Staphylococcus aureus* Pathogenicity Islands (SaPIs) are mobile genetic elements (MGEs) that encode virulence factors and depend on helper phages for their mobilization. They are clinically relevant because they play an important role in *S. aureus* pathogenicity. SaPIs are more than mere virulence gene carriers, they have developed different strategies that makes them critical in the evolution of the organism. For example, they mediate not only their own transfer but also they direct the horizontal transference of unlinked chromosomal DNA containing virulence genes. In addition, SaPIs can interfere with phage reproduction, increase host survival of phage infection or even force phage evolution.

Objectives: Due to this multifunctionality shown by the SaPIs, the aim of the study it to determine whether SaPIs could develope other functions important for bacteria and they could regulate the expression of chromosomal genes

Methods: Tilling array analysis and q-PCR of *S. aureus* strains with presence or absence of different MGEs.

Construction and characterization of *S. aureus* mutants

Results: Transcriptional analysis confirmed that SaPIs are involved in the regulation of host genes among which are operon *crtOPQMN* or acetyl transferase gene. We focused on study the regulation mechanism of chromosomal *crtOPQMN* operon which codify the carotenoid staphyloxanthin (STX). STX is a carotenoid pigment responsible for the characteristic golden color of *S. aureus* and it has been shown to be involved in bacterial virulence. Two SaPI proteins (PtiA and PtiM) were identified as responsible for STX overexpression and they seem to act by a novel antitermination regulatory mechanism.

PM332 The conserved phosphodiesterase PdeA contributes to the control of early growth of Brucella abortus in a trophoblast context of infection

Angéline Reboul[1], Phuong Thi Anh Ong[1], Xavier De Bolle[1]

[1]*University of Namur, Research Unit in Biology of Microorganisms, Namur, Belgium*

Background: *Brucellae* are facultative intracellular cocobacilli responsible for brucellosis, causing abortion and sterility. Animal trophoblasts are key cellular targets of the bacterium where they extensively replicate in the endoplasmic reticulum (ER), which is also the case in epithelial cells and in macrophages. However, in human trophoblasts, the niche of replication seems to vary depending on the cell type of trophoblasts. Bacterial replication occurs in ER compartment in cytotrophoblasts, mimicked by the BeWo cell line, but in large acidic inclusions, positive for the lysosomal membrane associated protein-1 (Lamp1), in extravillous trophoblasts, mimicked by the JEG-3 cell line (Salcedo *et al.*, 2013). Cyclic di GMP (cdG) is a major bacterial second messenger involved in numerous processes, such as acute-chronic infection transition, virulence, cell cycle control and bacterial growth.

Objectives: Our objectives are to discover if cdG influence *B. abortus* growth *in vitro* and in an infection context and to identify which cdG metabolizing enzymes are involved in this putative regulation.

Methods: We engineered a single mutant for each cdG metabolizing enzymes and monitored their growth during their first cell cycle thanks to unipolar growth and surface labelling *in vitro* and in infected cells.

Results: The analysis of the proportion of growing bacteria suggest that a phosphodiesterase mutant called *pdeA* was affected for the initiation of growth in JEG-3 trophoblast cells but not in RAW264.7 macrophages. This uncovers a putative role of cdG in the control of bacterial growth in specific intracellular environments.

PM333 Analysis of Causes and Consequences of Nosocomial Pneumonia in Military and Retired Personnel

Nataliia Popenko[1]

[1]*Ukrainian Military Medical Acadamy, Military Therapy, Kiyv, Ukraine*

Background: The level of mortality from nosocomial pneumonia (NP) increases dramatically with untimely or inadequate antibiotic therapy, bacteremia and if the etiological agent - P. aeruginosa or Acinetobacter spp.

Objectives: to investigate the causes of the occurrence, the peculiarities of the course, the range of pathogens and to assess the effectiveness of antibiotic therapy of NP in the military men and male pensioners.

Methods: A retrospective analysis of the causes and consequences of NP in 46 military men and male pensioners for the period of 2010-2015 was conducted. The average age of patients was 59.3 ± 2.9 years. Out of 46 patients, 25 (54.3%) people recovered, and 21 (45.7%) died. To identify the etiological structure of the NP, data from a bacterial study of sputum was used, which included the quantitative determination of the pathogen and its sensitivity to antibacterial drugs.

Results: Most often, NP was developed in patients with cardiovascular diseases (23,1%), respiratory diseases (chronic obstructive pulmonary disease, bronchial asthma) (22,6%), malignant neoplasms - 19,2%.

Representatives of the Staphylococcus family were isolated from the sputum of the examined patients in most cases - (30.0 ± 14.7) %. By frequency of detection, the next pathogens of the NP were representatives of the Enterobacteriaceae family - (17.5 ± 12.2) % and Pseudomonas aeruginosa - (17.5 ± 12.2) %. It was found that most NP pathogens were susceptible to ciprofloxacin (92.8 ± 10.0) % and ceftriaxone (89.3 ± 12.2) %.

The cause of deaths in most cases (61.9%) was acute cardiovascular insufficiency.

PM334 Molecular and biological features of avian pathogenic E. coli

Oleksii Nechypurenko[1], Iryna Sobko[2], Denys Dreval[3]

[1]*National University Kyiv-Mohyla Academy, Microbiology, Kyiv, Ukraine*
[2]*LLC "Centre of Veterinary Diagnostics", serology, kyiv, Ukraine*
[3]*LLC "Centre of Veterinary Diagnostics", Microbiology, kyiv, Ukraine*

Background: Avian pathogenic *E.coli*(APEC) is one of the main causes of economic losses in poultry industry. Obviously this bacteria is a secondary pathogen but in case of management predisposition and presence of virulence factors it can be a serious problem not only for poultry farming but to the general public health. It is known that APEC has a great genetic and structural similarity to extraintestinal humans *E.coli* that cause urinary infections, infants meningitis and sepsis.

Objectives: Investigate the molecular and biological features of APEC isolated from broilers, broiler's breeders and layers of Ukrainian poultry farms.

Methods: Routine autopsy and histopathology of birds with colibacillosis signs; isolation of bacteria from internal organs (spleen,liver,heart); identification of isolates using Api20E test, serotyping, PCR-detection of virulence genes, determination of susceptibility to antibiotics.

Results: It were identified 493 pathogenic *E.coli* strains (isolation from 2-3 organs/bird) among 548 isolated bacterial cultures from 513 birds during 2017-2018. Among APEC 47,5%, 9,9%, 4,6% of strains belong to O78, O2, O18 serogroup, respectively. There were not detected STa, STb, LT, Stx toxins at all. The prevalent virulence factors were iroN(94,5%), sitA(88,0%), iutA(78,9%), hlyF(74,8%), iss(58,0%). Also gentamicine, florfenicol, trimethoprim and ceftiofur were most effective antibiotics which killed 68 %, 59 %, 56% and 43% of totally isolated *E.coli* strains. However in 2018 resistance to colistin, trimethoprim and other systemic antibiotics increased reliably. Therefore it were selected several *E.coli* strains with prevalent serotype and combination of virulence factors, that supposed to be the candidates for autogenic vaccine against APEC.

PM335 Piscirickettsia salmonis infects and replicate inside the free-living protozoan Acanthamoeba castellani: a possible survival strategy in the environment

Martín Galaz[1], Bruno Milesi[1], Nicolas Ojeda[1], Sergio Marshall[1], Fernando Gomez[1]

[1]*Pontificia Universidad Catolica de Valparaiso, Instituto de Biología, Valparaiso, Chile*

Background: *Piscirickettsia salmonis,* a Gram-negative and facultative intracellular bacterium is the etiological agent of Piscirickettsiosis, a disease affecting the sustainability of salmon farming in Chile. Atpresent efficient control and prophylactic strategies are non-existing. Although the life cycle of *P. salmonis* has not been elucidated, the agent has been detected in native fishes but potential reservoirs or vectors have not been identified... Our work contributes to understand persistence of the bacteria in aquatic environments as a first step to design alternatives to avoid its spreading in salmon farms.

Objectives: To determine if *P. salmonis* could infect and survive in free-living protozoa, both in fresh and sea water, using Acanthamoeba *castellanii* as infection model.

Methods: An infection kinetics of the *Acanthamoeba castellanii* with an MOI of 10 of *P. salmonis of the* EM-90 genogroup was done at 2, 24, 48 and 168 h at 18ªC. Infection was confirmed by epifluorescence and Confocal microscopy. Functional expression of the bacteria was assessed by qRT-PCR of Dot/Icm secretion system genes.

Results: Our results demonstrate that *P. salmonis* infects *A. castellanii*, a fresh water amoeba. The bacteria were internalized through phagocytosis and enclosed inside digestives and contractile vacuoles at least for seven days in trophozoite and cyst stages of the amoeba. The expression of *dot/icm* genes confirmed the viability of *P. salmonis* inside the amoeba demonstrating an active infection. Our results suggest that *P. salmonis* could use marine amoeba as putative vector or reservoir in salmon farms, experiments in progress in our laboratory.

PM336 Influence of probiotics on Cronobacter sakazakii virulence

Kristina Schauer[1], Joyce Stecklum[1], Erwin Märtlbauer[1]

[1]Chair of hygiene and technology of milk, Faculty of Veterinary Medicine, Ludwig-Maximilians-Universität München, Oberschleißheim, Germany

Background: *Cronobacter sakazakii* are Gram-negative, facultative anaerobic bacteria of the family *Enterobacteriaceae* that have been implicated in rare but severe cases of illnesses predominantly in premature and new-born infants. This opportunistic pathogen has been isolated from clinical as well as from a range of food and environmental sources.

Objectives: Especially in new-born childrens, *C. sakazakii* infections have been epidemiologically linked to the ingestion of powdered infant formula (PIF). To reduce the risk of infection, probiotics should be tested as an addition to PIF.

Methods: Therefore, the inhibitory effect of different probiotic strains on *in vitro* growth of *C. sakazakii* and the ability to colonize human intestinal epithelial cells (Caco-2 and HT-29) was analyzed.

Results: The results show that co-infection with probiotic microorganisms protected the eukaryotic epithelial cells from invasion of the pathogen in a probiotic strain-dependent manner. Furthermore, with regard to the prophylactic effectiveness of probiotics, the inhibitory effect on *C. sakazakii* growth of sterile supernatants from probiotics in different PIF will be examined in order to developed approaches for safe and effective use of probiotics in PIF.

PM337 Pathogenic potential of Escherichia coli from various sources in Port Harcourt, Rivers State, Nigeria

Kome Otokunefor[1], Precious Nyema[1]

[1]*University of Port Harcourt, Microbiology, Port Harcourt, Nigeria*

Background: Despite its occurrence as a commensal, *Escherichia coli* is also notorious as a pathogen. One variation between these commensals and pathogens is the presence of specific factors one of which are the pathogenicity islands. One of the most commonly occurring of these is the PAI IV536. Potentially pathogenic species have been described in non-clinical settings. This often raises concerns on the role these environments play in transmission.

Objectives: This study therefore aimed at comparing the pathogenic ability of *E. coli* isolates from clinical and non-clinical sources based on the presence of the PAI IV536 marker.

Methods: Thirty-five *E. coli* isolates were analyzed in this study. Following DNA extraction by boiling, the PAI IV536 gene fragment was amplified following standard procedure using the F5'-AAGGATTCGCTGTTACCGGAC-3' and R5'-TCGTCGGGCAGCGTTTCTTCT-3' primer pair.

Results: Of the 35 isolates, 13 were from clinical sources and 22 from non-clinical sources. In total, 25.7% (9/35) of the *E. coli* isolates in this study were found to possess the PAI IV536 gene. Clinical isolates had a much higher association of 61.5% with the PAI IV536 gene than non-clinical which only had a 4.5% representation of the PAI IV536 gene.

This study reports on the detection of PAI IV536 in *E. coli* isolates from Port Harcourt, Rivers State Nigeria and a lower association of this pathogenicity marker with non-clinical isolates.

PM338 Searching new staphylococcal endolysins with potential antibacterial properties

Elías Cortés-Acosta[1;2], Mercedes Cervera-Alamar[1], Patricia Bernabé Quispé[1], Javier Pemán[1], Eulogio Valentin-Gomez[1;3], Mª Ángeles Tormo-Mas[1]

[1]*Instituto de Investigación Sanitaria La Fe de Valencia, Microbiology, València, Spain*
[2]*National Council for Science and Technology, Ciudad de México, Mexico*
[3]*Facultat de Farmàcia, Microbiología y Ecología, Burjassot, Spain*

Background: Antibiotic resistance is undoubtedly the greatest threats to global health. Infections caused by *Staphylococcus aureus* represents the most frequent causes of nosocomial infections, especially multidrug-resistant strains, such is the case of methicillin-resistant *S. aureus* (MRSA) and vancomycin-resistant, which are classified according to WHO as priority elevated regarding the need to obtain alternative treatments.

Endolysins are bacteriophage-encoded peptidoglycan hydrolases and they are produced at the end of the lytic cycle. They could access the peptidoglycan and destroy the bacteria. Recently it has been shown that they can be used as new therapeutic tools against bacterial infections without influencing the microbiota.

Objectives: The aim of the study is to discover and classify new *S. aureus* endolysins for the treatment of bacterial infections, in several clinical isolates. In addition, to test lytic activity of endolysins not previously studied.

Methods: 51 *S. aureus* clinical isolates were sequenced and analyzed to identify the presence of bacteriophages through integrases by an *in silico* analysis. Genetic mapping was done to identify endolysins-genes into bacteriophages sequences and were classified according to their catalytic and cell wall-binding domains, to later clone the most active proteins according to the *in vitro* previous analysis of these bacteriophages.

Results: 100% of the strains contained bacteriophage and 58.3% more than one. The endolysins were classify in 4 groups according at to catalytic sites. 86% of the strains have endolysins-phages with three domains CHAP/Amidase/SH. Until now we are cloning some endolysin genes to later purify them and test their lytic effect.

PM339 Bacteria in the Brain: the case of Eubacterium tarantellae

cesare montecucco[2], Luca Bano[3], Marco Pirazzini[1], Ullrich Dobrindt[4], Matthias Kiel[4], Gabriele Sales[5], Chiara Romualdi[5], Marco Schiavone[1], Andrew Doxey[6], Michael Mansfield[6;7], Ornella Rossetto[1]

[1]*Department of Biomedical sciences, university of Padova, padova,*
[2]*Department of Biomedical sciences, university of Padova, Department of Biomedical Sciences, University of Padova, padova, Italy*
[3]*Istituto Zooprofilattico Sperimentale delle Venezie*
[4]*Institute Hygiene, Universitat Munster*
[5]*Department of Biology, University of Padova*
[6]*Department of Biology University of Waterloo*
[7]*dep*

Background: *Eubacterium tarantellae* can be cultivated from the brain of apparently normal fish. It is capable of inducing twirling movements.

Objectives: to investigate *Eubacterium tarantellae* and how it alters fish.

Methods: the bacterium was originally isolated by Urey (1977) from both apparently healthy and twirling fish from Biscayne Bay (FL, USA). It was cultivated either in fortified cooked meat medium or in synthetic medium thioglycollate broth under anaerobiosis. It was subjected to SEM after fixation/dehydration and gold-shadowing. DNA was sequenced with the Illumina technology with a 60x coverage. DNA alignment and analysis was performed with the SPAdes software 3.10.1. The bacterium or its culture supernatant were injected in zebra fish or in mice using a stereotactic apparatus. Movement analysis was performed by videorecording.

Results: *E. tarantellae* is 20-40 µm long with diameters around 1 µm. 1.448.335 pair-end of DNA reads were assembled. The genome of *E. tarantellae* consists of nearly 4 million bases. It is about 40 % similar to *Clostridium perfringens* and 30 % to *Clostridium botulinum*. P*erfringolysin 0*, but no botulinum neurotoxin gene clusters, was present. The bacterium had no effect in mice, whilst in zebra fish it caused a characteristic twirling movement. The injection of the bacterial culture supernatants did not have any effect. On the contrary the injection of the supernatant of sonicated and centrifuged bacteria caused an almost immediate twirling behaviour; denaturation or trypsin treatments lowered the supernatant activity. We are currently attempting to isolate the neuroactive substance produced by *E. tarantellae*.

PM340 A rare case of Aerococcus viridans bacteraemia and neuroinvasive disease.

Andronikos Spyrou[1], Konstantinos Marousis[1], Eleni Karampatsaki[1], Georgios Plakias[2], Georgios Gkanteris[3], MYRTO ASTRITI[1]

[1]General Hospital of Athens "G. Gennimatas", 1st Department of Medicine & infectious Diseases Unit, Athina, Greece
[2]Hygeia, Microbiology department/Central Labs, Marousi, Greece
[3]General Hospital of Athens "G. Gennimatas", Department of Microbiology, Athina, Greece

Background: *Aerococcus viridans* has been commonly misidentified in routine laboratory practice. However, introduction of genomic and proteomic identification platforms has resulted in its recent increased isolation. Considered a contaminant in human cultures, *A. viridans* implication in invasive human disease has been questioned. Sparse reports hold *A. viridans* accountable for bacteraemia and to our knowledge, for only three cases of meningitis, in which, species was determined only by biochemical methods.

Objectives: To present the first case of *A. viridans* bacteraemia and meningitis in which pathogen isolation in the cerebrospinal fluid (CSF) was confirmed by Matrix Assisted Laser Desorption Ionization Time Of Flight (MALDI-TOF-MS).

Methods: Blood cultures were obtained with Bactec system. A lumbar puncture was performed before and during antimicrobial treatment. Culture products were identified by Vitec2 Compact System (BioMérieux, France) and CSF isolate was further analyzed with BioMérieux MALDI-TOF-MS. Antibiotic susceptibility was tested with Kirby-Bauer disk diffusion method and Vitek2.

Results: A cachectic 85-year-old woman with history of untreated diabetes mellitus, presented with altered mental status, fever, generalized seizures, nuchal rigidity and hypoxemia. Blood and CSF cultures were positive for *A. viridans* with confirmation by MALDI-TOF-MS analysis for CSF culture. Kirby-Bauer and Vitek2 revealed susceptibility to penicillin, vancomycin and linezolid. Repeated sets of blood cultures and a CSF culture, ten days after initiation of antimicrobial treatment with piperacilin-tazobactam were negative. A bone marrow aspiration and biopsy revealed serous atrophy, affirming the hypothesis of an immune-defective host. This case supports the conclusion of *A. viridans* pathogenicity at least in immunosuppressed individuals.

PM341 Western diet promotes gut colonization by vancomycin-resistant Enterococcus

Beatriz Herrera[1], Anna Quirant[1], María José Garzón[1], Sandrine Isaac[1], Carles Ubeda[1]

[1]*Fisabio, València, Spain*

Background: Antibiotic resistant pathogens (ARPs) such as vancomycin-resistant Enterococcus (VRE) are a major health problem. Infections with VRE frequently begin by colonization of the gut. The intestinal microbiota suppresses VRE colonization. Antibiotics enable VRE colonization by altering the microbiota composition. Other factors, recently introduced in our modern societies (i.e. western diet: rich on simple sugars and fat, low in complex carbohydrates) can alter the microbiota and therefore could enable VRE colonization.

Objectives: Investigate the effect of western diet induced dysbiosis on intestinal colonization by VRE.

Methods: A mouse model of infection and 16S-rRNA sequencing were used to investigate the effect of diet on VRE gut colonization.

Results: The majority (95%) of mice receiving a western-diet were colonized with VRE (on average 10^6 CFUs/g-faeces). In contrast, most mice fed with a control-diet (high in complex carbohydrates, low in fat) were resistant (18% colonized, $6x10^3$ CFUs/g on average). Western-diet induced major gut microbiota changes, including a decrease in richness and diversity. The degree of western-diet induced dysbiosis (bray-curtis distance to control mice) positively correlated with VRE colonization levels (spearman; rho=0.84; $p=2.9x10^{-5}$). Microbiota dysbiosis persisted 2 weeks after reintroducing a control diet which render mice partially susceptible to VRE colonization. In addition, antibiotic-treated mice, whose microbiota was reconstituted with faeces from western-diet fed mice were colonized with higher VRE levels than those reconstituted with faeces from control-diet fed mice.

Altogether, these results suggest that western-diet induced dysbiosis enhances gut colonization by a multidrug-resistant pathogen in the absence of antibiotic treatment.

PM342 Campylobacter jejuni from wild mouse (Micromys minutus); possibility of reservoirs for human campylobacteriosis

Junhyung Kim[1], Woohyun Kim[1], Jae-Uk An[1], Jae-Ho Guk[1], Soomin Lee[1], Seung-Hyun Mun[1], Hyokeun Song[1], Jun Gyo Suh[2], Je Kyung Seong[1;3], Seongbeom Cho[1;3]

[1]Seoul National University, BK21 PLUS Program for Creative Veterinary Science Research, Research Institute for Veterinary Science and College of Veterinary Medicine, Seoul, Korea, Rep. of South
[2]Hallym University, Department of Medical Genetics, College of Medicine, Chuncheon, Korea, Rep. of South
[3]Seoul National University, Korea Mouse Phenotype Center, Seoul, Korea, Rep. of South

Background: *Campylobacter jejuni*, is one of the most common zoonotic pathogens in worldwide. There have been a possibility that wild animals may affect the transmission of *C. jejuni*. However, there have been few studies on *C. jejuni* in wild mouse which have been known to be reservoirs of many zoonotic pathogens and have threatened human public health.

Objectives: The current study was conducted to analyze the characteristics of *C. jejuni* from wild mouse and to reveal the association with human campylobacteriosis.

Methods: First, we investigated the prevalence of *C. jejuni* in *Mus musculus* and *Micromys minutus*. Second, virulence/survival factors and antimicrobial resistance of *C. jejuni* isolates were investigated. Third, molecular epidemiological studies was performed to identify genetic relationships.

Results: This is the first study of *C. jejuni* in wild mice; particularly in possibility of reservoirs for human campylobacteriosis. The prevalence of *C. jejuni* in the *Micromys minutus* was 63.6% (42/66), while, 0% (0/49) in *Mus musculus*. When virB11 was positive in one isolate, all strains isolated from the same region showed the same positive results and vice versa. In addition, all isolates were susceptible to all antibiotics. In the multilocus sequence typing (MLST) analysis, only one newly defined sequence type (ST-8388) was identified and there was no genetic similarity between *C. jejuni* from wild mouse and from other sources including human. There is a possibility that the specific *C. jejuni* strain is continuously re-infected to another wild mouse or *C. jejuni* might be a normal intestinal flora in wild mouse.

PM343 A functional approach to evaluate the role of the two different Hfq proteins in Piscirickettsia salmonis virulence

Nicolas Ojeda[1], Fernando Gomez[1], Fabian Henriquez[1], Eduardo Venegas[1], Sergio Marshall[1]

[1]*Pontificia Universidad Catolica de Valparaiso, Instituto de Biologia, Valparaiso, Chile*

Background: *Piscirickettsia salmonis* is a highly virulent facultative intracellular and Gram-negative bacterium, that affects the sustainability of salmon production worldwide. To date, how virulence is regulated in this bacteria in completely unknown. Since in most pathogenic bacteria the Hfq protein associated with small RNAs (sRNAs) seem to play pivotal roles in the regulation of virulence and stress responses, we have recently described the presence of two Hfq-like genes (Hfq1 and 2) in *P. salmonis*, which are transcriptionally actives under different growth conditions and therefore we would like to define their role in gene expression regulation.

Objectives: Characterize the function of both Hfq variants of *P. salmonis,* their putative association with sRNAs and their involvement in virulence.

Methods: Expression of Hfq1 and Hfq2 under different growth conditions was determined by qRT-PCR and Western Blots. Polyclonal antibodies elicited against different epitopes of Hfq variants were used to immunoprecipitate Hfq- sRNAs complexes from liquid cultures and subsequent sRNA sequencing using the Illumina MiSeq plataform. Different *P. salmonis* Δ*hfq* mutants (for Hfq1 and Hfq2) are being generated to demonstrate the involvement of any of the two Hfq variants in virulence gene expression.

Results: Our result up to now show that both Hfq variants are fully expressed under different growth and stressing conditions. Hfq2 is highly conserved when compared to other Gram negative bacteria. Hfq1, although an SM-like protein sharing the sRNA binding domain with HFq2, is quite different in sequence from the latter. Notwithstanding both variants immunoprecipitate sRNA species which are now being sequenced.

PM344 The role of the EphA2 receptor in A. fumigatus infection

Esther Keizer[1], Han Wösten[1], Hans de Cock[1]

[1]*Utrecht University, Biology, Utrecht, Netherlands*

Background: Conidia of *A. fumigatus* are dispersed via the air and can reach the lungs. If not cleared the conidia can interact epithelial lung cells and activate an immune response and endocytosed. The β-1,3-glucan receptor EphA2 at the oral epithelium is involved in the proinflammatory immune response and endocytosis of *Candida albicans*.

Objectives: Investigate the involvement of EphA2 in internalization of conidia of *A. fumigatus* and initiation of the immune response.

Methods: The association and internalization of dormant, swollen or heat killed conidia with A549 type lung cells pretreated with or without an EphA2 kinase inhibitor was monitored by confocal microscopy. Induction of IL-8 production will be monitored using an ELISA.

Results: Association of conidia was not affected by the EphA2 inhibitor but heat-killed conidia associated less. Internalization of all forms was reduced by 50% indicating that EphA2 has a role in internalization but not association of *A. fumigatus* conidia.

PM345 Characterisation of two novel pemIK-family toxin-antitoxin systems present in plasmids carrying vancomycin resistance determinants in Staphylococcus aureus

Anna Madry[1], Benedykt Wladyka[1], Michal Bukowski[1]

[1]*Jagiellonian University, Department of Analytical Biochemistry, Faculty of Biochemistry, Biophysics and Biotechnology, Krakow, Poland*

Background: *Staphylococcus aureus* is one of the leading causes of bacterial infections worldwide. The threat posed by staphylococci is exacerbated by accumulation of antibiotic resistance determinants, often acquired on plasmids by horizontal gene transfer. Common elements in bacterial plasmids are toxin-antitoxin (TA) systems, which stabilise their maintenance. Recently, yet uncharacterised TA systems, *pemIK-Sa4* and *pemIK-Sa5*, were found on putatively plasmid contigs derived from vancomycin-resistant *S. aureus*. Sequence similarity to enterococcal plasmids suggestes that these TA systems could potentially contribute to trans-species acquisition of vancomycin resistance by the staphylococcal strains.

Objectives: The aim of this study was to investigate the two novel TA systems by examining the characteristic traits of functional toxins: ability to affect bacteria growth and RNase activity.

Methods: The coding sequences of the systems' components were cloned into expression vectors to assess their influence on bacteria growth. His-tagged toxins were produced and purified to examine their enzymatic activity towards MS2 phage RNA.

Results: The expression of PemK-Sa4 and PemK-Sa5 led to severe growth inhibition of bacterial cultures and both toxins exhibited RNAse activity, which clearly suggests that the toxin components are functional and may contribute to vancomycin resistance spread. The results suggest a potential role of the two newly discovered TA systems in dissemination of vancomycin resistance among different bacterial species. Importantly, such TA systems can promote maintenance of resistance determinants even in the absence of selective pressure.

The project was partially financed by statutory funds No. K/DSC/005452 of the Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University.

PM346 Bacteria associated to diarrheogenic Escherichia coli infections promotes Shiga toxin-producing Escherichia coli virulence

Mauricio Farfan[1], Joaquin Lopez[1], Mariana Izquierdo[1], Pablo Gallardo[1], Roberto Vidal[1], Juan Ossa[1], Mauricio Olivares[1]

[1]*Universidad de Chile, Santiago, Chile*

Background: Diarrheagenic *E. coli* (DEC) pathogenicity relies on the interaction of bacteria with the host's gut environment, which is controlled by the resident microbiota. In previous studies, we have identified indicative bacterial species of gut microbiota in DEC-positive diarrhea stool samples of children.

Objectives: To evaluate the role of *Citrobacter werkmanii* (CW) and *Escherichia albertii* (EA) in the pathogenicity of Shiga toxin-producing *Escherichia coli* (STEC)

Methods: RNA-seq analysis was performed in order to determine the effect of supernatant of CW and EA in STEC gene expression. T84 cells were infected with STEC in the presence or absence of supernatant of CW and EA and secretion of 38 inflammation markers was evaluated. Supernatants of CW and EA were analyzed by GC-MS to identify Short Chain Fatty Acids (SCFA)

Results: Supernatant of CW and EA has no effect in the growth of STEC and its ability to adhere to intestinal cells. RNA-seq analysis reveals that several STEC virulence factors were up-regulated in the presence of the supernatant tested. An increase in the secretion of IL-8 was observed in T84 cells infected with STEC in the presence of supernatant tested compared to cells infected with STEC alone or uninfected cells. GC-MS analysis reveals that formate and acetate are the main SCFA present in the supernatant tested. Our work provides new insights into the effect of resident gut microbiota species associated to DEC infection in the pathogenicity of STEC.

PM347 The role of T3SS effector YopE in adaptive immune response

Anna Lyapina[1], Valentina Feodorova[1], Maria Khizhnyakova[1], Sergey Zaitsev[1], Lidiya Sayapina[2], Maxim Telepnev[3], Onega Ulianova[1], Elena Lyapina[4], Sergey Ulyanov[5], Vladimir Motin[6]

[1]Federal Research Center for Virology and Microbiology, Branch in Saratov, Laboratory for Molecular Biology and NanoBiotechnology, Saratov, Russian Federation
[2]Scientific Center on Expertise of Medical Application Products, Department of Vaccine Control, Moscow, Russian Federation
[3]University of Texas Medical Branch, Galveston, TX, United States
[4]Saratov State Medical University named after V.I. Razumovsky, Department for Infectious Diseases, Saratov, Russian Federation
[5]Saratov State National Research University, Department for Medical Physics, Saratov, Russian Federation
[6]University of Texas Medical Branch, Department of Pathology, Department of Microbiology & Immunology, Galveston, TX, United States

Background: The bacterial type 3 secretion system (T3SS) is a potent virulence apparatus utilized by a wide spectrum of gram-negative pathogens. It acts like a syringe translocating the effector proteins into host target cells to interrupt their functioning, particulary dampening host immune response. Many T3SS components participate in inducing the protective antibacterial immunity. YopE is a T3SS effector, which plays a critical role in pathogenesis of yersinia infection due its antiphagocytic activity. It was shown to contain CD8+T cell specific epitopes protective in a mouse model.

Objectives: To characterize a role of YopE and a type (Th1/Th2/TH17) of induced homologous adaptive immune response in humans in a model of vaccine-elicited specific immunity.

Methods: A live plague vaccine (LPV) was used as the model bacterial vaccine possesing T3SS. The immunobloting technique was used to detect YopE-specific antibodies, while limphocyte proliferation assay (BrdU) and cytokine profiling (ELISA) were implemented to asses the type of YopE-specific immune responses in humans vaccinated with LPV versus naive donors.

Results: We showed that in vaccinated individuals YopE is involved in eliciting both humoral and cellular adaptive immune responses skewed to Th1-type with the production of specific antibodies and high levels of IFN-γ, TNF-α and IL-10 ($p<0.05$). Our data suggest that YopE may participate in protective anti-plague response in humans and have a potential for being a marker of the relevant immune response.

This study was supported by the RFBR #18-016-00159.

PM348 Molecular Identification of Chlamydia trachomatis Serovars in Urogenital and Ocular Samples

Iwona Lesiak-Markowicz[1], Anna-Margarita Schoetta[1], Gerold Stanek[1], Mateusz Markowicz[1]

[1]*Institute for Hygiene and Applied Immunology, Centre for Pathophysiology, Infectiology and Immunology, Medical University of Vienna, Vienna, Austria*

Background: The obligate intracellular bacterial pathogen *Chlamydia trachomatis* is known as the etiological agent of trachoma, oculogenital infectious disease, and lymphogranuloma venereum (LGV), linked to the serovars A-C, D-K, and L1-3, respectively. Currently, 19 human serovars and related variants of *C. trachomatis* have been identified. *C. trachomatis* infection is usually detected by nucleic acid amplification tests.

Objectives: Our aim was to determine the serovars in DNA extracts of original specimens (cervical and urethral swabs, urine or genital secretions, conjunctival swabs) which were sent to our laboratory in a four years period (2014-2017) and tested positive for *C. trachomatis*.

Methods: We used a method based on sequence analysis of the omp1 gene, encoding the major outer-membrane protein (MOMP).

Results: Among 401 positive samples (originating from 360 female and 19 male patients), serovar E was the most common one (50.1%), followed by serovars F (16.2%), D (9.7%), G (9.0%), J (6.0%), K (4.7%), H (2.7%), B (1.0%), and I (0.5%). We identified serotypes that are usually associated with urogenital tract infections (D-K) but one (B) that is linked to both, ocular and genital tract infection.

PM349 Contrasting patterns of infection by group A rotavirus and Shiga toxin-producing Escherichia. coli in young llamas and alpacas

Leonarda Acha[1], Ulises Caceres[2], Volga Iniguez[1], Elmer Pereyra[1], Julia Barreta[1], Lucia Gonzales Siles[3], Edward Moore[3]

[1]*Instituto de Biologia Molecular y Biotecnologia, Biology, La Paz, Bolivia*
[2]*Centro Experimental Agropecuario Condoriri., UTO, Oruro, Bolivia*
[3]*Institute for Biomedicine, Department of Infectious Diseases, Goteborg, Sweden*

Background: Acute gastroenteritis (AGE) in camelids is an important cause of disease and mortality. Group A rotavirus (RVA) and diarrheagenic *E.coli* (DEC) are considered to be the main diarrheal pathogens. AGE imposes severe economic losses in camelid production; therefore, it is critical to understand the epidemiology of enteropathogen infections.

Objectives: To describe the infections caused by enteropathogens (DEC and RVA) in young camelids and their relationships to AGE.

Methods: Fecal samples from 16 llamas and 11 alpacas were regularly collected from birth to weaning. Additionally, 21 stool samples were also collected from the rest of the flock, upon initiation of diarrheal symptoms.

Samples were analyzed for RVA by ELISA and for DEC by Polymerase Chain reaction, targeting virulence genes of the main DEC-pathotypes.

Results: DEC infections were found in asymptomatic animals, with STEC being the most prevalent pathotype with incidences of 55.1% and 81.0% in llamas and alpacas respectively. STEC infections were recurrent and long-lasting. Most of the STEC isolates were non-O157H7, carried predominantly the stx2 gene and were sensitive to all tested antibiotics except tetracycline. These data suggest that young domestic camelids are colonized by STEC early, during the first months of age.

RVA was found only in animals with diarrhea. An outbreak of acute diarrhea occurred in the cold season, affecting 27 llamas and 5 alpacas. RVA (G3 genotype) was identified in 93.1% of llamas and 80.0% of alpacas.

These data show contrasting patterns of RVA and STEC in the relationships between infection and AGE in young camelids.

PM350 The cell death of lung fibroblasts by live Mycobacterium tuberculosis bacilli infection specifically induces inflammatory cytokines

Takemasa Takii[1;2], Saotomo Itoh[2], Shigeaki Hida[2], Kikuo Onozaki[2]

[1]Research Institute of Tuberculosis, Japan Anti-Tuberculosis Association, Mycobacteriology, Kiyose, TOKYO, Japan
[2]Nagoya City University, Graduated School of Pharmaceutical Sciences, Nagoya, Aichi, Japan

Background: Different types of cell death, apoptosis and necrosis, is proposed to term as pyroptosis. The cell death after infection of pathogenic bacilli is thought to induce the inflammatory reaction in host by producing various inflammatory mediators. We have previously reported that human lung fibroblast cell lines, MRC-5, -9, and TIG-1, were killed by live Mycobacterium tuberculosis (MTB) bacilli.

Objectives: In order to analyze the physiological role of fibroblasts at MTB infection to host, the induction activity of mRNA of inflammatory cytokines was investigated in human fibroblasts infected with MTB bacilli.

Methods: Human fibroblast cell line, MRC-5, was incubated with M. tuberculosis H_{37}Rv. The mRNA expression of cytokines was measured by RNase protection assay. The transcription activity interleukin (IL)-6 and IL-8 gene in the infected fibroblasts was measured by reporter assay using luciferase gene.

Results: The mRNA expressions of IL-6 and IL-8 in MRC-5 were augmented 2 days after infection with live MTB bacilli, but not with dead bacilli. The transcriptions of IL-6 and IL-8 genes were simultaneously induced in the cells by the infection. The cell death of infected cells was also observed 2 days after the infection. These results suggest that the cell death of fibroblasts would augment the inflammatory reaction in MTB infection. These cytokines from lung fibroblasts induced by MTB infection may play an important role in the progression of lung tissue damage in tuberculosis.

PM351 Bacterial co-infection of diarrhoeagenic escherichia coli pathotypes and salmonella species among gastroenteritis patients in some selected pastoral hinterlands of the amathole district municipality, eastern cape, south africa.

Luyanda Msolo[1;2], Anthony Ifeanyi Okoh[2;3]

[1]SA-MRC Microbial Water Quality Monitoring Centre, University of Fort Hare, Biochemistry and Microbiology
[2]Applied and Environmental Microbiology Research Group, Biochemistry and Microbiology, Alice, South Africa
[3]SA-MRC Microbial Water Quality Monitoring Centre, University of Fort Hare, Biochemistry and Microbiology, Alice,

Background: Diarrhoea as the consequence of Gastroenteritis is one of the most significant causes of infant's deaths across the universe. Over 700 000 deaths of children less than 5 years of age occur each year as a result of gastroenteritis infection**.**

Objective: The main objective of this study was to investigate the potential bacterial aetiological agents of diarrhoea within the selected rural settlements of the Amathole District Municipality, Eastern Cape, South Africa.

Methods: Standard culture-based techniques were used for enumeration of diarrhoeagenic *E. coli* (DEC) pathotypes and *Salmonella* species from diarrhoeal stool samples using relevant selective media. Polymerase Chain Reaction (PCR) technique was used to confirm the identities of the presumptive bacterial agents recovered from diarrhoeagenic stool specimens.

Results: A total of 208 (64 %) isolates were confirmed by PCR as DEC and were further classified into 4 DEC pathotypes and an additional 116 (36%) isolates were confirmed as Non-diarrhoeagenic *E. coli.* Among the confirmed DEC pathotypes, Enterotoxigenic *E. coli* (ETEC) (51 %) was the most prevalent followed by Diffuse adherent *E. coli* DAEC (18%), Enteroaggregative *E. coli* (EAEC) (16%) and Enteropathogenic *E. coli* (EPEC) (15%). Subsequently; 62 (23%) of 263 presumptive *Salmonella* isolates were also confirmed by conventional Polymerase Chain Reaction (PCR) using genus specific primer sets. The findings of this study indicate that co-infection of bacterial pathogens such as DEC and *Salmonella* species are one of the most common features in the aetiology of diarrhoea.

PM352 Confocal microscopy shows hilD allelism role in Salmonella Derby intracellular replication phenotype within human enterocytes

Melissa Berni[1], Martina Tambassi[1], Silvana Belletti[2], Stefano Guizzardi[2], Stefano Pongolini[1], Gabriele Casadei[1]

[1]*Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Risk Analysis and Genomic Epidemiology Unit, Italy*
[2]*University of Parma, Department of Medicine and Surgery, Parma, Italy*

Background: Within the~10.000 *Salmonella* isolates collection at the Emilia Romagna Regional Enteropathogens Reference Center, the two most prevalent *Salmonella* Derby Pulsed-Field Gel Electrophoresis (PFGE) profiles in swine have a different prevalence in human: PFGE_Hu profile is equally isolated in the two hosts, while PFGE_Sw profile is significantly less isolated in human than in swine. We identified a single-nucleotide polymorphism (SNP) in *hilD*, a positive transcriptional regulator of Salmonella pathogenicity island-1 (SPI1), as a genetic determinant responsible for lower invasion efficiency and intracellular-growth rate of PFGE_Sw isolates, compared to PFGE_Hu isolates, in human cells.

Objectives: Within mammalian cells, pathogens can replicate in *Salmonella*-containing vacuole (SCV) or hyper-replicate colonizing the cytoplasm. Since we demonstrated a significant difference in intracellular-growth rate between PFGE_Hu and PFGE_Sw isolates, we sought to microscopically observe the replication phenotypes of *S.* Derby within human intestinal epithelial cells (INT407).

Methods: We performed the gentamicin protection assay on INT407 cells infected with *S.* Derby isolates representative for PFGE_Hu and PFGE_Sw transformed with mEGFP-pBAD plasmid. In order to observe intracellular pathogens, monolayers were fixed, then stained with Evans Blue for fluorescence-confocal imaging.

Results: In PFGE_Hu infected monolayers, confocal images showed epithelial cells with a high load of intracellular pathogens, indicative of cytoplasm colonization, while in PFGE_Sw infected cells this phenotype was not observed, consistently with SPI1 involvement in vacuolar escape and cytosolic replication. Further investigations are required to understand the role of *hilD*-dependent replication phenotypes in *S.* Derby host tropism.

PM353 Functional characterization of Type III effectors repertoire of Pseudomonas syringae pv. actinidiae Biovar 3 that can suppress yeast viability in stress conditions

Oriana Flores[1], Mayra Mérida[1], Isabel Rodríguez-Escudero[2], María Molina Martín[2], Víctor Jiménez Cid[2], Dionisia Sepúlveda[3], Víctor Cifuentes[3]

[1]Pontificia Universidad Católica de Valparaíso, Laboratorio de Microbiología, Instituto de Biología, Facultad de Ciencias, Valparaíso, Chile
[2]Universidad Complutense de Madrid, Departamento de Microbiología II, Facultad de Farmacia, Madrid, Spain
[3]Universidad de Chile, Laboratorio de Genética, Departamento de Ciencias Ecológicas, Facultad de Ciencias, Ñuñoa, Chile

Background: *Pseudomonas syringae pv. actinidiae* (Psa) is causal agent of bacterial canker of kiwifruit that has caused severe losses in Italy, China, New Zealand and Chile. Psa-V group include the most virulent strains, which have a specific type III effector proteins repertoire that would play a important role in bacterial pathogenicity.

Objectives: We identified and characterized functionally Psa-V putative effectors proteins in yeast heterologous expression system previously used to study bacterial effector target in cellular processes conserved in eukaryotes.

Methods: Effector genes (40 in total) were identified in genome of Chilean Psa-V isolates by bioinformatic analysis, which 31 were cloned in Galactose inducible yeast plasmid. Viability assays, cell fitness, morphological characterization by fluorescence microscopy and cell wall integrity (CWI) and high osmolarity glycerol (HOG) pathways activations were analyzed in yeast transformants.

Results: Different effectors tested displayed growth inhibition or lethal phenotypes in yeast. Cell fitness, actin cytoskeleton, mitochondria distribution and endosomal trafficking analysis, suggest that effector no altered this yeast components or cell process. However, using phosphorylation reported, we found effector genes can attenuated the activation of CWI and HOG under prolonged exposition to stress inductor. Interestingly, despite the others effector proteins, as HopX3, can reduce yeast viability in osmotic stress conditions, not alter HOG pathway phosphorylation, suggesting that these proteins have different targets. This is the first study about functionally of Psa type III effectors effector in yeast and provide signs of effector cell target. Funding. CONICYT grants FONDECYT Postdoctorado 2017 No. 3170567.

PM354 Assessment of Antimicrobial Susceptibility Profile of Gram Positive Bacteria of semen Samples

Alvard Hovhannisyan[1], Inessa Nazaryan[2], Narek Pepanyan[2], Anna Karapetyan[2], Oksana Hovhannisyan[2], Naira Gyulazyan[1]

[1]*Yerevan State Medical University, Infectious Diseases, Yerevan, Armenia*
[2]*Ecosense Diagnostic Center, Yerevan, Armenia*

Background: Antibiotic resistance is rising to dangerously high levels in all parts of the world. In the evaluation of a genitourinary tract infection (GTI), semen culture is considered an important diagnostic tool as genitourinary tract infection are potential causes for male infertility.

Objectives: Study aims to assess antimicrobial susceptibility profile of gram-positive bacteria collected from semen samples of patients with GTI.

Methods: Total of 150 semen samples from different hospitals of Armenia were collected from the patients with suspected GTI. The VITEK® 2 microbial testing system was used for microbial identification and antibiotic susceptibility testing. Data entered into a dedicated database (Microsoft excel). Descriptive analysis was computed for all variables of interest.

Results: Microbial identification results of 74 (49%) samples were positive for gram-positive bacteria and 50 (33%) for gram-negative bacteria. In 18% of cases no bacteria were detected.

Antimicrobial susceptibility profile of gram-positive bacteria was presented in Table 1.

Table 1.

Gentamicin	71%	**Co-trimoxazole**	54%
Cefuroxime	98%	**Ampicillin/sulbactam**	94%
Clindamycin	79%	**Amoxicillin /clavulanic acid**	94%
Ceftriaxone	99%	**Ofloxacin**	84%
Doxycycline	83%	**Ciprofloxacin**	89%
Moxifloxacin	92%	**Amoxicillin**	75%
Levofloxacin	93%	**Clarithromycin**	77%
Azithromycin	70%	**Tetracycline**	56%

Conclusion: Low susceptibility of gram-positive bacteria was revealed for Co-trimoxazole and Tetracycline (54% and 56% accordingly). The identification of the critical points provides necessary support to devise better strategies for treatment. Also, assessing the level of antimicrobial drug resistance offers accurate information to formulate more efficient antibacterial protocols rationalize the use of antibiotics.

PM355 Outer membrane vesicles in Francisella tularensis - characterization and role in host-pathogen interaction

Jan Bavlovič[1], Jana Klimentová[1], Ivona Pávková[1], Lenka Plzáková[1], Klára Kubelková[1], Magdaléna Prokšová[1], Jiří Stulík[1]

[1]*University of Defence, Faculty of Military Health Sciences, Department of Molecular Pathology and Biology, Hradec Králové, Czech Republic*

Background: Francisella tularensis is a non-motile gram-negative coccobacillus, a highly virulent intracellular pathogen and a potential biological warfare agent. Bacterial outer membrane vesicles (OMVs) are particles of spherical shape of 20-200 nm, which are formed by the bulging and separation of part of the bacterial outer membrane. OMVs are produced by all gram-negative bacteria. OMVs represent one of the main ways of secretion that serves for the interaction with both other bacterial and eukaryotic cells. Excretion of vesicles is generally considered as an adaptive response to stress and OMVs also may have several tasks during the infection.

Objectives:

- Study of the OMVs morphology in F. tularensis and its mutants with disrupted surface structures.
- Proteomic analysis of isolated OMVs.
- Characterization of their role in host-pathogen interaction on cellular level (cytokine release, cytotoxicity and influence of the bacterial entry into macrophages).

Methods: TEM and SEM, fluorescent microscopy, proteomic analysis, cytokine quantification

Results: F. tularensis forms OMVs of unique nanotubular shape. Their proteomic analysis revealed number of virulence factors and immunostimulatory proteins. Within the set of mutants with disrupted surface structures we detected big differences in the shape and size of the vesicles. Morphology of the whole bacteria showed importance of lipopolysaccharide and polysaccharide capsule for tubular protrusions. OMVs treatment had no significant influence on the bacterial entry into macrophages. Nevertheless, rapid entry of isolated OMVs into macrophages was observed together with prolonged viability and pro-inflammatory cytokine response in the host cells which suggests their role in host-pathogen interaction.

PM356 Involvement of the RND efflux pump transporter SmeH in the acquisition of resistance to ceftazidime in Stenotrophomonas maltophilia

Paula Blanco[1], Fernando Corona[1], Jose Luis Martinez[1]

[1]*Centro Nacional de Biotecnología, Microbial Biotechnology, Madrid, Spain*

Background: The emergence of antibiotic resistant Gram-negative bacteria has become a serious global health issue. *Stenotrophomonas maltophilia* is an opportunistic nosocomial pathogen responsible for causing a variety of infections in patients with underlying pathologies, as cystic fibrosis, and in those who are immunocompromised. One of the main characteristics of this bacterium is its intrinsic low susceptibility to a broad range of antibiotics, which entails a difficulty in the treatment of the infections that it causes.

Objectives: The aim our study is to use *S. maltophilia* as a model to determine the genetic mechanism involved in the acquisition of mutation-driven resistance to the third-generation cephalosporin ceftazidime.

Methods: Four *S. maltophlia* independent cultures were serially passaged during 30 days in the presence of increasing concentrations of ceftazidime. The genetic mechanisms involved in ceftazidime resistance were determined through genomic DNA extraction and Illumina sequencing.

Results: We determined that exposure to increasing concentrations of ceftazidime selects high-level resistance in *S. maltophilia* through a novel mechanism: amino acid substitutions in SmeH, the transporter protein of the resistance nodulation division (RND) family SmeGH efflux pump. The recreation of these mutants in a wild-type background demonstrated that, in addition to ceftazidime, the existence of these substitutions provides bacteria with cross-resistance to other beta-lactam drugs. This acquired resistance does not impose relevant fitness costs when bacteria grow in the absence of antibiotics. Structural prediction of both amino acid residues points that the observed resistance phenotype could be driven by changes in substrate access and recognition.

PM357 Detection of genetic virulence markers in Escherichia coli isolates obtained from clinical bovine mastitis in Brazil

Rodrigo Tavanelli Hernandes[1], Henrique Orsi[1], Simony T. Guerra[1], Sâmea Fernandes Joaquim[1], Felipe Freitas Guimarães[1], Simone B. Lucheis[1], José C. de Figueiredo Pantoja[1], Márcio G. Ribeiro[1], Helio Langoni[1], Vera L. M. Rall[1]

[1]São Paulo State University (UNESP), Botucatu, Brazil

Background: Bovine mastitis comprises the most common disease in dairy cattle worldwide, and *Escherichia coli* is one of the most commonly pathogens associated with bovine mastitis of environmental origin. Nevertheless, to date, the genetic profile of *E. coli* isolates associated with bovine mastitis is still poorly understood.

Objectives: The objective of this study was to define the genetic virulence profile of *E. coli* isolates obtained from cows diagnosed with clinical mastitis, in 10 Brazilian farms.

Methods: A total of 142 *E. coli* isolates were tested for the presence of 34 virulence-encoding genes, 13 from diarrheagenic (DEC) and 21 from extraintestinal pathogenic (ExPEC) *E. coli*, using Polymerase Chain Reaction.

Results: Based on the presence of DEC markers, only one (0.7%) atypical enteropathogenic *E. coli* (*escN*$^{+}$/*bfpA*$^{-}$/*stx*$^{-}$) was identified. Moreover, 4 isolates (2.9%) were positive for *aaiA*, and 8 isolates (5.6%) were positive for *astA* (EAST1 enterotoxin), indicating that they may belong to the EAEC (enteroaggregative *E. coli*) pathotype. On the other hand, isolates harboring genes encoding for adhesins (*fimH*=99.1% and *ecpA*=64.5%), toxins (*hlyA*=6.4%, *vat*=2.1% and *cdt*=2.1%), iron acquisition (*irp2*=8.5%, *sitA*=7.9% and *iroN*=2.8%), invasin (*ibe10*=1.4%) and protectins (*traT*= 81.8%, *ompT*=36.9% and *kpsMTII*=3.0%) were also detected. In conclusion, our data reveal that the *E. coli* isolates studied rarely carry genes encoding virulence factors associated with the establishment of diarrheal disease in humans. Instead, a wide range of virulence genes implicated in extraintestinal infections were detected, thus confirming the pathogenic potential of these isolates. (Financial support: FAPESP/2015/19688-8).

PM358 Anti-inflammatory activity of Beta-caryophyllene combined eith docosahexaenoic acid in a model of sepsis induced by Staphylococcus aureus in mice

Deborah Cruz dos Santos[1], Lais Ferraz[1], Hellen Braga M Oliveira[1], Nathan das Neves Selis[1], Clarissa Leal Souza e Silva[1], Manoel Neres Santos Júnior[2], Erika Pereira de Souza[1], Lucas Coelho Santana Silva[2], DEBORAH CRUZ DOS SANTOS[1], Regiane Yatsuda[1], Guilherme Barreto Campos[2], Lucas Miranda Marques[1;2;3]

[1]*Universidade Federal da Bahia (UFBA) - Campus Anísio Teixeira, Brazil*
[2]*Uesc – Universidade Estadual De Santa Cruz, Brazil*
[3]*University of São Paulo, São Paulo, Brazil*

Background: Sepsis is a set of serious organic manifestations caused by an infection, whose progression culminates in exacerbated inflammation and oxidative stress, poor prognosis, and high hospital costs. Antioxidants have been evaluated against sepsis, including essential oils, such as β-caryophyllene (BCP), and polyunsaturated fatty acids, such as Docosahexaenoic Acid (DHA).

Objective: The aim of this study was to evaluate the anti-inflammatory activity of the association of these two compounds.

Methods: Forty-eight male Balb/c mices were used. First, we realized the Carrageenan-induced peritonitis assay for anti-inflammatory activity screening . Later, the animals were inoculated with *Staphylococcus aureus*, euthanized after 24 and 48 hours. Fluids and tissues were extracted for total and differential count of leukocytes, cytokines dosage, histological and bacterial analyzes.

Results: Treatment with BCP-DHA, at a dose of 200 µL/animal, significantly inhibited the migration of neutrophils in a Cg-induced peritonitis model. After *S. aureus* infection, in the groups treated with BCP-DHA, there was a significant decrease in the total and differential count of leukocytes; increased expression of cytokines TNF-α and IFN-γ in treated groups, rise of IL-4 and IL-5 in B/D and B/D + SA groups and augment of IL-6 and IL-12 groups in B/D + SA groups. Histological and bacterial analysis revealed lower neutrophil migration and lower bacterial load, in the infected and treated groups.

PM359 The organised trip of Klebsiella pneumoniae

Sylvie Miquel[1], Laurence Nakusi[1], Christiane Forestier[1]

[1]*Université Clermont Auvergne, UMR CNRS 6023 LMGE, Clermont Ferrand, France*

Background: Nosocomial infections with *Klebsiella pneumoniae* constitute a major public health problem, with severe pulmonary infections. The reservoir of this bacterium is the intestine and infection of the patients' organs occurs via exogenous or endogenous ways. In this latter case, it has been shown that the bacteria reach the lungs after crossing the intestinal epithelial barrier, but the molecular process has not been elucidated.

Objectives and results: *In vitro* study of the interactions of *K. pneumoniae* with both intestinal and pulmonary epithelial cells indicated that *K. pneumoniae* has a high tropism for lung epithelial cell A-549. This adhesion was not due to the production of type 3 pili by the bacteria and did not alter the integrity of the cell layer as measured by determination of the transepithelial electrical resistance (TEER). On the opposite, adhesion of *K. pneumoniae* to Caco-2 intestinal epithelial cells considerably affected the integrity of the cell layer with a TEER decrease. Modification of the tight junctions were also observed such as delocalization of the ZO-1 protein and over-expression of the pore-forming protein Claudine 2.
These results need to be confirmed in an *in vivo* model where the local microbiota could influence the crosstalk between the host and the pathogen.

PM360 Brucellosis in wildlife and pets in Finland - One health aspects

Varpu Hirvelä-Koski[1], Teresa Skrzypczak[2]

[1]*Finnish Food Authority, Research and Laboratory Services Department, Oulu, Finland*
[2]*Finnish Food Authority, Veterinary Bacteriology and Pathology Unit, Helsinki, Finland*

Background: The source of human *Brucella* infection is always live or dead animals or contaminated food of animal origin. Human cases are usually caused by *Brucella melitensis*, *B. abortus* and *B. suis* biovar 1 and 3 harbouring sheep, goats, cattle and domestic swine. Due to effective eradication programs, livestock in Finland is free from brucellosis. However, during the past few years *Brucella* infections have been detected in Finnish wildlife as well as in imported dogs.

Objectives: The goal of this poster is to highlight the recent findings of *Brucella* infections in Finnish dogs and wildlife and to increase the awareness of the possibility of autochthonous infections in human beings.

Methods: Examinations for *Brucella* infections were performed using serological and cultivation methods.

Results: *Brucella suis* biovar 2 was for the first time found in the year 2016 in wild boars (*Sus scrofa*). Of 206 animals, 5 % were positive serologically and 3 % by the cultivation method.

Brucella pinnipedialis was isolated for the first time in the year 2013 in grey seals (*Halichoerus grypus*) at the coastal area of the Baltic Sea.

Brucella canis has been isolated in imported dogs.

Even though these *Brucella* species are regarded as low-pathogenic to human beings, all of them have also been reported to cause serious disease in humans.

PM361 Modulation of virulence in Legionella pneumophila: story of a mobile genetic element round trip

Guillaume Carrillo[1;2], Christophe Ginevra[1], Sophie Jarraud[1], Vincent Daubin[2], Elisabeth Kay[1]

[1]*Centre International de recherche en infectiologie, Villeurbanne, France*
[2]*Laboratoire de biométrie et biologie évolutive, Villeurbanne, France*

Background: The *Legionella* genus comprises mainly waterborne, facultative intracellular bacteria that replicate within a wide variety of protists. When inhaled by Humans, *Legionella* infect pulmonary macrophages, resulting in a potentially fatal pneumonia called Legionnaire's disease. Comparative genomics revealed that interkingdom gene transfers that occurred during the coevolution of *Legionella* with its multiple eukaryotic hosts shaped the bacterial genome and probably provided substantial "weapons" allowing *Legionella* to become a Human pathogen.

Objectives: We address the question of the virulence robustness and host spectrum in *Legionella*. We wondered whether bacteria evolving in the absence of hosts for hundreds of generations lose or retain their ability to infect protists and mammalian cells.

Methods: We conducted a mutation accumulation evolution experiment in which several replicate populations, founded from a *L. pneumophila* clinical isolate, were serially propagated on standard agar medium.

Results: After several hundred generations of genetic drift, evolved clones exhibited a decreased ability to infect both amoebae and human macrophages compared to the ancestor. This intracellular growth defect was correlated with a reduced capacity to establish the replication-permissive vacuole although the type 4 secretion system, which is the key element of virulence in *Legionella*, was still functional. Using whole genome sequencing, we have identified all the genomic modifications accumulated during evolution. We especially characterized one mutation shared by all the independently evolved lineages that confers the attenuated virulent phenotype. Ultimately, we demonstrated that this mutation and the associated-phenotypes can be reversed when the attenuated virulent clone was re-evolved in a more constrained environment.

PM362 Distinct transcriptional programs underlie differences in virulence of isolates on host plants in a fungal pathogen, Colletotrichum gloeosporioides

Younmi Lee[2], Wonsu Cheon[3], Young Soo Kim[1], Junhyun Jeon[4], Yongho Jeon[1]

[1]Andong National University, Plant medicals, Andong, Korea, Rep. of South
[2]Agricultural Science and Technology Research Institute, Andong National University, Andong, Korea, Rep. of South
[3]Nakdonggang National institute of Biological Resources, Sangju, Korea, Rep. of South
[4]Yeungnam University, Biotechnology, Gyeongsan, Korea, Rep. of South

Background: During our survey of apple anthracnose, we recognized that there are two distinct types of disease symptoms, which we designated as progressive (PS) and static symptoms (SS).

Objectives: Experiments were conducted to determine the cause of the both symptoms.

Methods: The causal organisms of the two pathogens were isolated, and the biological characterization and RNA-seq analysis were performed.

Results: PS is a typical, rapidly enlarging symptom of apple anthracnose, while SS is a small, dark speck that does not expand further until harvesting season. Difference in symptom development was independent of temperature and fruit maturity. Isolation and genotyping of pathogens from disease lesions suggested that all of them are *Colletotrichum gloeosporioides.* Two isolates were comparable in growth on media, spore germination and appressorium formation. However, SS isolate was defective in appressorium-mediated penetration into underlying substratum. Interestingly, we found that SS isolates are more similar in their symptom development and genotype to an isolate from acacia tree (AS), which is commonly found in the periphery of apple orchards, than to PS isolate. RNA-seq analysis of three isolates showed that distinct transcriptional programs underlie development of different types of anthracnose symptoms on host plants. Transcriptome profiles of SS and AS isolates were similar to each other, while that of PS isolate was distinct from the two. Taken together, our work not only challenges traditional view on development of different lesion types but also provide insight into variations that exist in seemingly identical pathogen population.

PM363 A Salmonella virulence protein activates pho regulon to stimulate phosphate transport

Soomin Choi[1], Eun-Jin Lee[1]

[1]Korea University, Life Sciences and Biotechnology Division of Life Sciences, Seoul, Korea, Rep. of South

Background: A *Salmonella* enterica serovar Typhimurium is an intracellular pathogen that causes typhoid fever, paratyphoid fever, and food poisoning. *Salmonella* has a unique lifecycle that survives within a macrophage phagosome. Within the phagosome, *Salmonella* senses low Mg2+, low pH, and antimicrobial peptides as signals to express virulence genes. Phosphorus is one of the essential elements in living organisms. Phosphate is a component of DNA, RNA, ATP, or phospholipids. In *Salmonella*, expression of pho regulon involved in phosphate transport is controlled by the PhoB/PhoR two-component system. The PhoB / PhoR two-component system regulates phosphate transport through the interaction with the Pst high-affinity phosphate transporter complex and the PhoU regulator.

Objectives: We wondered if effects of phosphate transport function on *Salmonella* when it infected with host. In addition, we wanted to figure out correlation of virulence genes of *Salmonella* and phosphate trasport system, *pho* regulon.

Methods: We used bacterial two hybrid and immunoprecipitation to detect protein interactions, gentamicin protection assay to measure replication efficiency within macrophages.

Results: Here we found that a *Salmonella* virulence protein stimulates expression of pho regulon and activates phosphate transport by a direct interaction. We determined key residues for the interaction and the substitution studies suggest that the interaction is required for *Salmonella* pathogenesis.

PM364 Investigating the impact of bile on Salmonella Paratyphi virulence

Elli Mylona[1], Dereck Pickard[2], Gordon Dougan[2], Stephen Baker[3;4], Avinash Shenoy[5], Gad Frankel[1]

[1]*Imperial College London, Department of Life Sciences, London, United Kingdom*
[2]*Wellcome Trust Sanger Institute, Cambridge, United Kingdom*
[3]*Oxford University Clinical Research Unit, Hospital for Tropical Diseases, Ho Chi Minh City, Viet Nam*
[4]*Oxford University, Centre for Tropical Medicine and Global Health, Nuffield Department of Clinical Medicine, Oxford, United Kingdom*
[5]*Imperial College London, Department of Medicine, London, United Kingdom*

Background: Human infection with *Salmonella* Paratyphi leads to typhoid fever. Tyhpoid causes more than 11 million cases and 200,000 deaths worldwide annually. While *S*. Paratyphi accounts for fewer typhoid cases than other typhoidal serovars, its incidence has been on the rise. However, pathogenesis of *S*. Paratyphi remains poorly studied. During infection, *S*. Paratyphi encounters bile in the small intestine and the gallbladder upon systemic dissemination. While the effect of bile on other *Salmonella* serovars virulence has long been recognised, it has not been investigated in the context of infection with *S*. Paratyphi.

Objectives: The aims of this study were to investigate *S*. Paratyphi pathogenesis through its response to bile and its interaction with macrophages.

Methods: The *S*. Paratyphi response to bile was analysed by RNAseq on bacteria grown in the presence or absence of 3% ox-bile and validated with real time PCR. A reference sequenced strain was used, alongside a clinical isolate, currently used in human challenge studies. Interactions of *S*. Paratyphi and THP1 cells, a macrophage-like cell line, was investigated through measuring *S*. Paratyphi-induced cell death and activation of innate immune pathways.

Results: We found that *S*. Paratyphi growth was not affected following exposure to bile. Unexpectedly, in the presence of bile the glyoxylate shunt, which is important in acetate metabolism, was downregulated, a response that appears to be *S*. Paratyphi-specific. As this pathway has been shown to be involved in persistent macrophage infection with other bacteria, we are exploring its role during macrophage infection with *S*. Paratyphi.

PM365 A small ORF controls translation of the Mg2+ transporter in Salmonella enterica

Eunna Choi[1]

[1]*Korea University, Life Sciences, Seoul, Korea, Rep. of South*

Background: A *Salmonella enterica* serovar Typhimurium is an intracellular pathogen whose ability to survive within a phagosome inside host macrophages is critical to cause disease. Mg^{2+} is an abundant and essential ion in living cells. This cation is involved in many important biological processes including coordinating nucleotides, stabilizing ribosome or membrane, and participating in many enzymatic reactions. In *Salmonella,* Mg^{2+} transport is controlled by three distinct Mg^{2+} transporters: MgtA, MgtB and CorA.

Objectives: We found that a short ORF located upstream of the Mg^{2+} transporter. The location of the ORFs are conserved among several bacteria. We studied how short OFR regulate translation of the Mg^{2+} transporter.

Methods: We constructed the nucleotide substitutions in the *mgtB* leader were constructed by cloning PCR fragment generated by two rounds of PCR reactions. A plasmid vector harbors the fusions to a promoterless gfp gene. The recombinant plasmids confirmed gfp expression.

Results: These results show that a nucleotide substitutions revealed that the ORF controls translation of the Mg^{2+} transporter

PM366 Determination of susceptibility to antimicrobials in staphylococci isolated from people living in rural settlements

Michel Ullofo do Nascimento[1], Rafael Silva Rosa[1], Gabrielle Messias de Souza[1], João Vitor Lapa Guarnier[1], Maria Clara Antunes Alves[1], Gabriel Henrique Maximino Santos[1], Diego Júnior Santos Gonçalves[1], Giovana Nascimento Pereira[1], Lizziane Kretli Winkelstroter Eller[1], Luiz Euribel Prestes Carneiro[1], Valeria Cataneli Pereira[1]

[1]*UNOESTE, Microbiology, Presidente Prudente, Brazil*

Background: Agrarian reform in Brazil resulted in regions known as rural settlements, which concentrate a large number of individuals living in poor housing and hygiene conditions, being vulnerable to multiple infectious agents, presenting recurrent cases of staphylococcal infections.

Objectives: The study aimed to determine the susceptibility to antimicrobials in isolates of *Staphylococcus s*pp. from the nasal cavities and the oropharynx of residents of a rural settlement.

Methods: Clinical-epidemiological data were collected from individuals younger than 18 years and older than 60 years. Bacterial samples were collected from the nasal cavities and oropharynx through a swab inoculated in Baird Parker Agar for selection of *Staphylococcus* spp. *Staphylococcus* isolates were submitted to the diffusion disc test to determine antimicrobial susceptibility.

Results: Samples were obtained from 74 participants, where 81.1% were under 18 years and 18.9% were over 60 years. These individuals live in the family environment and only 17.3% reported attending the urban area daily while 25.7% attend weekly, 29.7% fortnightly and 27.0% monthly. In the last year, 62.1% reported upper respiratory infections, 13.5% had skin infections, 12.5% hospitalization and 40% had antimicrobial use. Of the 148 samples, 28.10% were isolated from *Staphylococcus* spp. and 55.5% of staphylococci were resistant to erythromycin, 71.1% to penicillin, 60% to clarithromycin, 31.1% to oxacillin and 11.1% to cefoxitin. The data showed colonization by multiresistant staphylococci by these vulnerable individuals who live isolated from the urban area, sharing fomites and being able to easily disseminate these bacteria that can cause infections of skin and upper airways.

PM367 Effect of UVC radiation on pathogens in situ and in wound infections

Elias Hakalehto[1;2], Anneli Heitto[1], Ilkka Hirvonen[3], Urho Väätäinen[3]

[1]*Finnoflag Ltd, Siilinjärvi, Finland*
[2]*University of Helsinki, Department of Agricultural Sciences, Helsinki, Finland*
[3]*Led Future Ltd, Varkaus, Finland*

Background: Surgical patients need to be protected against infectious agents during operations. In order to optimize safety, ultraviolet C (UVC) could be used for local restriction of spreading of pathogenic microbes into the wounds and lesions. Pathogenic microbes may have more than one cellular form, or growth mode or phase, which depend on the surrounding milieu and have an influence on the UVC susceptibility of the strains. Also such variables as pigmentation, influence the outcome of the targeted UVC treatments.

Objectives: The purpose of the experimentation was to develop methods for measuring specific effects of the UVC on the various microbial strains, with and without the contact to the eukaryotic cells or tissues. This included the susceptibilities of different microbes in variable metabolic and environmental conditions.

Methods: We used precisely-controlled LF WCD (Wound Cleaning Device)™ equipment for exact dosing of UVC light on pathogenic bacteria. Typical exposure times of 0, 20, 60 and 120 sec were applied with different wave lengths. Microbial growth after the UVC treatments was measured by the PMEU (Portable Microbe Enrichment Unit) cultivation unit.

Results: Impacts of UVC radiation on the attenuation of microbial growth were dependent on several environmental factors as well as strain related features, besides the intensity and duration of the treatments. Our method produced growth curves of the microbes. The protocol turned out to be useful for finding effective and safe levels of UVC doses for microbiological patient protection. Susceptibilities between strains could vary more than average effects between microbial genera.

PM368 Active chromatin modifications induced by streptococcus pneumoniae alter inflammatory responses

Wenyang Dong[1], Melanie Hamon[1]

[1]*Institut Pasteur, Department of Cell Biology & Infection, Paris, France*

Background: Bacterial pathogens reprogram host cells during infection and studying these processes is central to the understanding of infectious diseases. One such mechanism of host reprogramming is through bacteria triggered histone modifications, which modulates the host's transcriptional programs.

Objectives: The goals of this study are to uncover new mechanisms of host cell transcriptional reprogramming, and to understand the role certain histone modifications play during bacterial infection.

Methods: We use *Streptococcus pneumoniae* as a model bacterium to explore the cross talk between host chromatin and a pathogen of the respiratory tract. An *in vitro* infection model using human lung epithelial cell line, and a mouse infection model are established to study histone modifications induced by *S. pneumoniae.*

Results: The *in vitro* infection model shows that *Streptococcus pneumoniae* infection leads to dephosphorylation of histone H3 on serine 10. We have identified at least 2 bacterial factors important for inducing this modification: the bacterial toxin PLY, a pore-forming toxin which is released upon bacterial autolysis, and the pyruvate oxidase SpxB, an enzyme responsible for H2O2 production. PLY and H2O2 from *S. pneumoniae* lead to host signaling culminating in H3S10 dephosphorylation, a process that we show to be mediated by the host cell phosphatase PP1. Importantly for infection, we find that H3S10 dephosphorylation correlates with a decreased expression of certain inflammatory genes, and decreased neutrophils and NK cell recruitment *in vivo*. In conclusion, we show that *S. pneumoniae* modifies host chromatin using multiple factors, which results in altered inflammatory responses.

PM369 Detection of Bartonella spp. by PCR in blood samples of warty patients negative for Bartonella bacilliformis.

Miguel Angel Aguilar-Luis[1;2], Hugo Carrillo-Ng[2], Wilmer Silva-Caso[2;3], Jorge Bazán-Mayra[4], Victor Zavaleta-Gavidea[4], Carmen Tinco-Valdez[2;3], Juana del Valle-Mendoza[2;3]

[1]School of Medicine, Research and Innovation Center of the Faculty of Health Sciences. Universidad Peruana de Ciencias Aplicadas, Lima, Peru
[2]Laboratorio de Biologia Molecular, Instituto de Investigación Nutricional., Lima, Peru
[3]School of Medicine, Research and Innovation Center of the Faculty of Health Sciences. Universidad Peruana de Ciencias Aplicadas, Lima, Peru
[4]Laboratorio Regional de Cajamarca, Dirección Regional de Salud de Cajamarca (DIRESA), Cajamarca, Peru

Background: *Bartonella bacilliformis* is a gram negative, facultative intracellular bacteria, responsible for a varied spectrum of disease which, has been given multiple names including Bartonellosis, Carrion's disease, Oroya fever and verruga peruana. This infection is endemic in low-income Andean areas of Peru. This organism is responsible for two different clinical syndromes. An acute phase characterized by fever and haemolytic anemia and a subsequent phase, characterized by skin lesions or verrugas ("warts").

Objectives: The aim of this study was to determine the prevalence of *Bartonella bacilliformis* in febrile patients from Cajamarca, Peru.

Methods: We included patients who presented to outpaint health centers with acute febrile illness, defined as an axillary temprature greater or equal to 38°C in the previos 7 days, without an identificable source of infection. Patients who had received treatment before consult, patients with an incomplete medical record and patients with an identificable source of infection were excluded. Real-time PCR was performed for the detection of *Bartonella bacilliformis* and Conventional PCR for the detection of *Bartonella* spp.

Results: We included 138 patients in our study, of which 13 patients had Peruvian wart and in a first diagnosis they were negative for *Bartonella bacilliformis* and *Bartonella* spp. The samples were stored for 12 months and after this time the presence of the aforementioned agents was analyzed. The results show 4 (30.76%) positive cases for *Bartonella* spp. and no positive case for *Bartonella bacilliformis*.

PM370 Estrogen reverses the age-related impaired clearance of wound bacteria by human macrophages via activation of estrogen-receptor alpha and actin cytoskeleton reorganisation

Jason Ashworth[1], Kathryn Whitehead[1], Mohamed El Mohtadi[1]

[1]*the School of Healthcare Science, Manchester Metropolitan University, Manchester, United Kingdom*

Background: Chronic wounds often become colonised by opportunistic pathogens such as *Staphylococcus aureus* and *Pseudomonas* aeruginosa. Estrogen deprivation with increasing age delays healing but the effect on wound infections is largely unknown.

Objectives: To investigate the effect of hormonal aging (estrogen deprivation) on host clearance of methicillin-resistant *S. aureus* (MRSA) and *P. aeruginosa*.

Methods: U937- and human peripheral blood monocyte-derived macrophages were used in phagocytosis assays (n=24) to determine bacterial clearance following treatment with estrogen at levels found in elderly ($<1x10^{-9}$M) and young adults ($1x10^{-8}$M), and following exogenous supplementation ($1x10^{-7}$M). The mechanisms by which estrogen mediates phagocytosis were interrogated using estrogen receptor (ER) agonists/antagonists followed by immunoblotting to assess downstream effects on mediators of the actin cytoskeleton.

Results: Estrogen-deprivation significantly ($P<0.05$) inhibited the clearance of MRSA and *P. aeruginosa* compared to physiological (typical of youth) or supraphysiological levels. Confocal microscopy confirmed estrogen deprivation reduces macrophage uptake of fluorescent GFP-*S. aureus* and mCherry-*P. aeruginosa*. Stimulation of phagocytosis following ER-alpha (ER-α) activation was completely reversed by ER-α antagonism ($P<0.05$), whereas ER-beta (ER-β) activation had no effect on bacterial clearance. Scanning electron microscopy confirmed estrogen induces bacterial internalisation through dynamic formation of pseudopodia by phagocytes and this was mirrored by elevated levels of focal adhesion kinase (FAK), Rac-1, Cdc-42 and Rho-G but reduced levels of Rho-A. These findings suggest estrogen deprivation with increasing age leads to impaired bacterial clearance and that novel dressings that provide estrogen supplementation or selective activation of ERα may be effective treatment options for colonised wounds in the elderly.

PM371 In-vivo antibacterial impact of fiscus exasperata, securinega virosa and tamarindus indica leaf extract on bacterial isolates from otitis media effusion

Abidemi Ojo[1], olugbenga anthony ojo[2;2], Seun Owolabi Adebajo[1;3], abiola oladotun[3;3;3;4;4;4], ganiyat sodunke[3;4]

[1]federal university of agriculture, abeeokuta, nigeria, department of microbiology, abeokuta, Nigeria
[2]madona university, elele, port harcourt, dept of pharmacognosy, faculty of pharmacy, Nigeria
[3]federal university of agriculture, abeeokuta, nigeria, department of microbiology
[4]federal university of agriculture, abeeokuta, nigeria

Background: Antibiotics resistance threats in the treatment of Otitis media effusion continue to be a major challenge in the clinical settings.

Objectives: The aim of this study was to determine the efficacy of three medicinal plant extract in the treatment of Otitis media as an alternative therapy

Methods: The antibacterial effect of methanol leaf extract of *Ficus exasperate, Securinega virosa,* and *Tamarindus indica* on organisms isolated from otitis media effusion was determined using agar well diffusion method and the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) was also determined using the double fold dilution technique.

Results: Bacteria isolated include *Staphylococcus aureus* 19 (39.58%), *Klebsiella pneumonia* 15 (31.25%), *Pseudomonas aeruginosa* 10 (20.83%), *Proteus mirabilis* 3 (6.25%) and *Streptococcus pneumoniae* 1 (2.08%). Highest inhibition was recorded against *Pseudomonas aeruginosa* with a zone of inhibition value of 14.17 mm at 1.0mg concentration of *Ficus exasperata.* At 1.0mg concentration, *Tamarindus indica* was able to inhibit all the isolates*. Ficus exasperata* has least MIC of 0.39mg/ml and MBC of 0.78mg/ml against *Pseudomonas aeruginosa*. *Securinega virosa* has least MIC of 0.78mg/ml and MCB 1.56mg/ml against *Staphylococcus aureus, Pseudomonas aeruginosa* and *Klebsiella pneumoniae. Tamrindus indica* has least MIC of 0.39mg/ml and MBC of 1.56mg/ml against *streptococcus pneumoniae*. *Proteus mirabilis* was least inhibited with MIC values 50mg/ml, 50mg/ml and 25mg/ml for *Ficus exasperata, Secrinega virosa* and *Tamarindus indica* respectively with no MBC value for all the three extracts. The antimicrobial activity of the three extracts shows significant ($P<0.05$) inhibition for the organisms.

PM372 Gene transfer and association of antibiotic resistance genes among phage susceptible Acinetobacter baumannii

Udomluk Leungtongkam[1], Rapee Thummeepak[1], Sutthirat Sitthisak[1;2]

[1]*Naresuan University, Microbiology and Parasitology, Phitsanulok, Thailand*
[2]*Naresuan University, Centre of Excellence in Medical Biotechnology, Faculty of Medical Science, Phitsanulok, Thailand*

Background: *Acinetobacter baumannii* is an important pathogen that has developed antibiotic resistance and posies a major threat to public health. Antibiotic resistance genes can be spread by horizontal gene transfer mediated through bacteriophages.

Objectives: We determined the antibiotic resistance patterns and phage susceptibility among 230 *A. baumannii* clinical isolates and investigated the antibiotic resistance gene transfer using *in vitro* transduction.

Methods: A total of 230 *A. baumannii* strains were identified and we determined the antibiotic susceptibility patterns. Six *A. baumannii* bacteriophages were isolated from waste water and the susceptibility of different *A. baumannii* phages was ascertained by using the spot test. *In vitro* transduction was studied using extensively drug-resistant *A. baumannii* (XDR-AB) and *A. baumannii* isolated from environment.

Results: The pervalence of multi-drug resistant *A. baumannii* (MDR-AB), carbapenem resistant *A. baumannii* (CR-AB) and XDR-AB was 86.52%, 83.49% and 12.17%, respectively. *A. baumannii* resistant to tetracycline ($P < 0.001$) and harbored *tet*(B) gene ($P = 0.004$) were found to be associated with phage susceptibility. Transduction demonstrated that AB019 bacteriophage able transduce *tet*(B) gene from AB135 into both recipients; NU013R and NU015R. To the best of our knowledge, this is the first study reporting antibiotic resistance gene transfer in *A. baumannii* using *in vitro* transduction. This information is crucial for preventing and controlling the dissemination of antibiotic resistant genes in the environment.

PM373 Lytic DNA viruses from Methanogens

Mei-Chin Lai[1], Wen-Hsin Wei[1], Wei-Ling Zhang[1], Chieh-Yin Weng[1], Sheng-Chung Chen[1]

[1]*National Chung Hsing University, Department of Life Sciences, Taichung, Taiwan*

Background: Currently, over 100 archaeal viruses have been discovered, most related to the thermophilic Crenarchaeota and extreme halophilic Euryarchaeota. Knowledge of virus in strict anaerobic methanogens is very limited. Here, we introduced two novel lytic DNA viruses isolated from marine methanogens *Methanoculleus taiwanensis* and a novel genus and new species of family Methanocellaceae strain CWC-04, respectively.

Objectives: Isolation, characterization and genomic analysis of marine methanogen virus VMta (Virus of Methanoculleus taiwanensis) and VMce (Virus of Methanocella).

Methods: Strict anaerobic Hungate technique for cultivate methanogenic cultures; TEM (JEM-1400, JEOL); PEG precipitation and FPLC separation for virus purification; Illumina MiSeqTM genome sequencing.

Results: Both marine methanogen virus VMta (Virus of Methanoculleus taiwanensis) and VMce (Virus of Methanocella) are dsDNA virus with spherical virion, about 100 nm for VMta and 170 nm for VMce; with icosahedral capsids and envelope. VMta contained 70,262 bp with a mol% G+C content of 54.5 and 101 putative open reading frames (ORFs). Genome analysis suggested VMta may injected the DNA into the host by the puncture protein and replicated their genomes through the rolling-circle replication (RCR) mechanism. The viral DNAs were packed into the empty capsid by the terminase enzymes and virons left host cell with the endolysin disruption. Both M. taiwanensis and Methanocellaceae strain CWC-04 were isolated from the deep sea gas (methane) hydrate habitat, the lytic virus may play a role in deep sea carbon cycle.

PM374 Small terminase subunit from bacteriophage G18

Evgeny Klimuk[1;2], Alfred Antson[3], Konstantin Severinov[1;2]

[1]*Skolkovo Institute of Science and Technology, Moskva, Russian Federation*
[2]*IMG RAS, Moscow, Russian Federation*
[3]*University of York, Heslington, United Kingdom*

Background: Viruses are the most abundant biological agents on Earth, they infect organisms of all domains of life. Encapsidation of genome is essential part of viral life cycle and in many cases is mediated by specialized molecular machines fuelled by energy derived from macroergic compounds hydrolysis. While there is significant progress in getting new structural and functional data for such molecular machines encoded by bacteriophages, a number of important questions about the mechanism of DNA packaging into viral particles remain open, for instance - about DNA recognition and initiation of packaging process for tailed bacteriophages.

The main problem in the field is based on instability of these complex multisubunit molecular machines.

Objectives: To address these issues we conduct structural and functional characteristics of the DNA packaging motor from recently isolated bacteriophage G18 infecting thermophilic bacterium Thermus thermophilus. Increased stability and robustness of proteins from thermophilic sources open new horizons in studying viral DNA packaging.

Methods: Bioinformatic analysis of bacteriophage G18 genome; cloning of genes encoding for components of the DNA packaging motor; heterologous expression and tandem purification of resulting proteins; crystallization; 3D structure determination; ChIP; NGS; creation of phage G18 mutants.

Results: Cluster of genes encoding for DNA packaging motor components of bacteriophage G18 was predicted, genes were cloned to expression vectors. Resulting proteins were purified to homogeneity, tested for oligomeric state and used for crystallisation. 3D structure of small terminase subunit was determined. Policlonal antibodies against small terminase subunit were purified for ChIP-experiments. Mutant G18 phages were created.

PM375 Structural studies of adenovirus binding to integrins

Aleksandra Stasiak[1], Thilo Stehle[1]

[1]*Interfakultäres Institut für Biochemie, Eberhard Karls Universität Tübingen, Tübingen, Germany*

Background: Human adenoviruses (HAdVs) are non-enveloped viruses with a double-stranded DNA genome of about 35 kilobases. As pathogens, they are associated with gastrointestinal or respiratory symptoms, and conjunctivitis, among others. Moreover, they are one of the most-studied gene delivery vectors for vaccination, gene, and cancer therapy.

For successful entry into the host cell, HAdVs require binding to both an attachment and an entry host receptor, interacting with the viral capsid proteins of the fibre knob and the penton base, respectively. Research in our laboratory has previously focused on defining interactions of the fibre with attachment receptors such as Coxsackievirus and Adenovirus Receptor (CAR), CD46 and sialic acid.

Objectives: The current project aims to examine the viral interaction with host cell entry receptors from the integrin protein family and to solve a high-resolution structure of the penton-base-integrin-complex.

Methods: X-ray crystallography is a powerful technique for resolving interprotein interactions up to an atomic level of detail. High-quality crystals and large amounts of proteins of interest are required for solving a structure, so a variety of protein production and purification methods will be used. Alternative methods such as cryo-electron microscopy will also be considered.

Results: A structure of the virus-entry receptor complex will enhance our understanding of early stages of HAdV infection, which could inform rational design of drugs against HAdV as well as the development of targeted gene delivery vectors.

PM376 Monitoring biological soil crust reactivation by flow cytometry to set up virome analysis

Gianmarco Mugnai[1], Milda Stuknytė[1], Stefania Arioli[1], Alessandra Adessi[2], Diego Mora[1]

[1]University of Milan, Department of Food, Environmental and Nutritional Sciences (DeFENS), Milan, Italy
[2]University of Florence, Department of Agricultural, Food, Environmental and Forestry Science and Technology (DAGRI), Florence, Italy

Background: Biological soil crusts (BSCs) are one of the dominant community types on Earth, composed of micro and macro poikilohydric organisms. Particularly in drylands, BSCs play a vital role in biogeochemical cycles and geomorphological processes, mainly due to the microbial activity, which are reactivated only upon moisture uptake. In these communities, the diversity and role of viruses remain poorly understood.

Objectives: The aim of this study was to reactivate the BSCs in order to isolate the associated virus-like particles (VLPs).

Methods: An innovative protocol was developed for the BSCs reactivation monitoring. It was based on flow cytometry (FCM) and a biological marker: the amount of live cells released from BSCs after a controlled hydration and day-light exposure. Living microbial cells were monitored by FCM after a SYTO™ 24 and propidium iodide (PI) dual staining and a carboxyfluorescein diacetate succinimidyl ester (CFDAse) single staining. The reactivated BSCs were subjected to the isolation of VLPs through a tangential flow filtration and cesium chloride ultracentrifugation steps, followed by DNA extraction suitable for shotgun metagenomic virome analysis.

Results: An innovative FCM-based protocol was for the first time applied for the BSCs reactivation monitoring. It was useful for the isolation of VLPs from BSCs samples. The results obtained allowed the isolation of 200 ng of viral DNA from 300 g of BSCs. The controlled BSCs reactivation protocol developed in this study will be useful in all the studies focused on the characterization of microbial activities of BSCs.

PM377 A P1/P7-like prophage mediating transmission of an incorporated transposon comprising a blaCTX-M-15 resistance gene

Claudia Jäckel[1], Valerie Osieka[1], Alexandra Irrgang[1], Silvia Schmoger[1], Annemarie Käsbohrer[1;2], Jens André Hammerl[1]

[1]German Federal Institute for Risk Assessment, Department of Biological Safety, Berlin, Germany
[2]University of Veterinary Medicine, Institute for Veterinary Public Health, Vienna, Austria

Background: P1-/P7-like prophages were supposed as efficient vehicles for the transfer of antimicrobial resistance determinants. The transfer of antimicrobial resistances by phages may represent an evolutionary adaption to extend the number of possible intra- and interspecies hosts.

Objectives: To characterize the genetic basis of ESBL-producing *E. coli* isolates and its mechanisms for the transmission of the cephalosporin antimicrobial resistance, *Escherichia coli* isolates recovered from wildlife in Germany between 2015 and 2017 were characterized in detail. Within this study, an *E. coli* isolate was identified harboring a P1-/P7-like prophage comprising a cephalosporin resistance determinant.

Methods: The isolate was studied by S1-PFGE, DNA-DNA hybridization and WGS analysis to determine the genetic basis of the cephalosporin resistance. Restriction profiling, transformation, *in vivo* filter-mating and antimicrobial resistance testing was performed to characterize the properties of the isolate and its mobile genetic elements. Mitomycin C inductions were conducted to assess the activity of the prophage and its impact for the transfer of antimicrobial resistances.

Results: Genome determination of the *E. coli* isolate revealed a $bla_{\text{CTX-M-15}}$ carrying sequence contig exhibiting significant homologies to known P1-/P7-like plasmid prophages. Induction and plaque tests indicated that the phage represents a broad host range. The phage showed a morphology of typical myoviruses and is able to infect *E. coli* strains of different serotypes. Due to the incorporation of the phage in novel recipient *E. coli*, lysogenic conversion of the bacteria was observed by the production of extended-spectrum beta-lactamases.

PM379 Evaluation of the elution buffer and concentraction methods for recovering norovirus from various fruits

JeongSu Lee[1], jinhee hwang[1], eunsook an[1], minhee jeong[1], ara jo[1], hyosun kwak[1]

[1]national institute of food and drug safety evaluation, division of food microbiology, cheongju, Korea, Rep. of South

Background: Norovirus (NoV) outbreaks due to consumption of fruits, vegetables, and shellfish have been reported during recent years. The detection of NoV in food could be difficult and is mainly defendant on food matrix.

Objectives: This study aimed to compare the elution buffers and concentration methods for detecting NoV from fruits such as apple, grape, and strawberry.

Methods: Twenty-five gram fruit samples were artificially inoculated with murine NoV 1 (MNV-1). Spiking experiment with MNV-1 was conducted to determine the efficiency of viral elution-concentration methods. The inoculated virus on each fruit was washed using 0.25M threonine-0.3M NaCl (pH 9.5), TGBE buffer (100mM Tris-HCl, 50mM glycine, 3% beef extract (pH 9.5)), 0.25 M glycine-0.3M NaCl (pH 7.5), and phosphate buffered saline (pH 7.5). As the next procedure, viral concentration was conducted using ultrafiltration (UF) and polyethylene glycol (PEG). The QIAamp viral RNA mini kit was used to extract RNA from concentrated viruses, according to manufacturer's instruction. Real-time reverse transcription-polymerase chain reaction (RT-PCR) was performed to detect MNV-1 RNA.

Results: Finally, we found to be the most efficient methods for the elution and concentration of NoV from fruits. The best methods to detect NoV from fruits were TGBE-ultrafiltration for apple, strawberry and TGBE-PEG for grape. The average recovery rate of viral elution-concentration methods for apple and strawberry were 59.2% and 24.2% by TGBE-ultrafiltration, respectively. The average recovery rate of grape was 71.7% by TGBE-PEG. These methods could assist to trace the foodborne outbreaks and to identify the possible source of NoV contamination.

PM380 Mutaion and genetic polymorphism of varicella-zoster virus passaged in cell culture under unnatural conditions

Chan Hee Lee[1], Sang Hoon Yeon[1], Hye Rim Hwang[1], Se Hwan Kang[1], Jeong Seon Jeon[1]

[1]*Chungbuk National University, Microbiology, Cheongju-si, Korea, Rep. of South*

Background: Varicella-Zoster virus (VZV) is a member of herpes virus and contains double-stranded DNA genome. Primary infection of VZV causes varicella and often leads to zoster after reactivation from latency. Both varicella and zoster can be prevented by live attenuated vaccines, but the molecular mechanisms are not exactly understood.

Objectives: It was attempted to understand mechanism of attenuating mutation in VZV by in vitro passaging under unnatural culture conditions such as low temperature or xenogenic cell.

Methods: VZV strain YC02 was passaged under natural (37C, human cell) and unnatural (34C, guinea pig cell) conditions up to passage 100. Genome sequences of selected passages were determined by next generation sequencing (NGS).

Results: The infectivity of YC02 cultured under natural condition increased in high passages, but YC02 cultured under unnatural conditions exhibited decreased rate of infectivity. NGS of the YC02 genome cultured under various conditions identified mutations specific for passagingof the virus in low temperature (34C) and xenogenic cell (guinea pig lung fibroblast) culture. The number of genetically polymorphic sites where the minor allele frequencies exceed 5% increased when culture under natural condition, but did not increase when cultured under unnatural conditions employed in this study, suggesting a bottleneck event during the evolution of the virus. Further studies on mutation and genetic polymorphism inder unnatural conditions will help to understand the molecular mechanism of VZV attenuation and evolution.

PM381 Biodiversity and full genome sequence of potato viruses alfalfa mosaic virus and potato leaf roll virus in egypt

Engy Essam Abdel Aleem Awad[1], Radwa Taha[2], Faiza Fattouh[3]

[1]Faculty of science - alexandria university- egypt, Botany and Microbiology Department, alexandria, Egypt
[2] Faculty of Science, Damanhour University, Damanhour, Egypt, Botany Department, Damanhour, , Egypt
[3] Faculty of Science, Alexandria University, Alexandria, Egypt, Botany and Microbiology Department, Alexandria, Egypt

Background: *Solanum tuberosum* (potato) is the second most important vegetable crop in Egypt. It is locally consumed, manufactured or supplied for export to Europe and other Arab countries.

Objectives: Potato is subject to infection by a number of plant viruses, which affect its yield and quality. *Potato virus Y* (PVY), *potato leaf roll virus* (PLRV), and *Alfalfa mosaic virus* (AMV) were detected in major potato-growing areas surveyed.

Methods: Multiplex-RT-PCR assay was used for the detection of these three viruses in one reaction using three specific primer pairs designed to amplify genomic parts of each virus. Full length sequencing was performed for one isolate of each of the viruses.

Results: All three viruses were detected in a single reaction mixture in naturally infected field-grown potatoes. Multiplex RT-PCR improved sensitivity necessary for the early detection of infection. Incidence of single, double, or triple infection has been recorded in some locations. Full length sequencing has been performed for an Egyptian FER isolate of PLRV. Through phylogenetic analysis, it was shown to occupy the same clade with isolate JokerMV10 from Germany. Complete nucleotide sequence of an Egyptian FER isolate of AMV and phylogenetic analysis was also performed; we propose that it is a new distinct strain of AMV belonging to a new subgroup IIC. This is the first complete nucleotide sequence of an Egyptian isolate of AMV. Genetic biodiversity of devastating potato viruses necessitates continuous monitoring of new genetic variants of such viruses.

PM382 The specificity of yeast totiviruses and mechanisms behind it

Lina Aitmanaitė[1], Aleksandras Konovalovas[1], Povilas Medvedevas[1], Elena Servienė[2;3], Saulius Serva[1;3]

[1]*Vilnius University, Institute of Biosciences, Vilnius, Lithuania*
[2]*Nature Research Center, Laboratory of Genetics, Vilnius, Lithuania*
[3]*Vilnius Gediminas Technical University (VGTU), Department of Chemistry and Bioengineering, Vilnius, Lithuania*

Background: Yeast L-A viruses belong to *Totiviridae* family, observed among *Saccharomycetales* and other yeasts. Some strains together with L-A helper virus carries satellite dsRNA M virus, expressing a killer phenotype. DsRNA genome of L-A encodes major capsid protein Gag and a fusion protein Gag-Pol - RNA dependent RNA polymerase, crucial for maintenance of L-A and M viruses in a cell. Satellite (M) dsRNA genome encodes a secreted protein toxin. L-A spreads by direct cytoplasm contact, extracellular stage of the virus remains unidentified. Different L-A viruses are associated with specific M viruses.

Objectives: With this study, we focused on the complex interactions between different L-A and M viruses to get a deeper understanding of mechanisms responsible for observed specificity. For that, we constructed expression vectors of different wild type and chimeras proteins of L-A viruses.

Methods: The impact of expressed recombinant proteins on maintenance of intrinsic L-A and M viruses was accessed by viral genomic dsRNA analysis and killer phenotype assays.

Results: We demonstrate that over-expression of capsid protein alone has minimal impact on native L-A viruses, while Gag-Pol over-expression has a significant impact and depends on the type of native L-A virus. The same trend is observed for the killer phenotype. Over-expression of chimera proteins provided data on mechanisms and specific regions of Gag and Gag-Pol proteins tentatively responsible for the specific interactions between L-A and M viruses.

PM383 Characterization and Diversity of Temperate Bacteriophages Induced in Pasteurella multocida from Different Host Species

Rezheen F. Abdulrahman[1;2], José R Penadés[2], Robert Davies[2]

[1]*Collage of Veterinary Medicine, University of Duhok, Pathology and Microbiology Department, Duhok, Iraq*
[2]*Institute of Infection, Immunity and Inflammation, College of Medical, Veterinary and Life Sciences, University of Glasgow, Bacteriology Department, Glasgow, United Kingdom*

Background: *Pasteurella multocida* is a Gram-negative commensal bacterium which resides in the upper respiratory tract of mammals and birds. It causes a variety of economically important diseases in a wide range of domestic animals including fowl cholera of poultry, haemorrhagic septicaemia of cattle and water buffalo, atrophic rhinitis of pigs, and pneumonia of cattle, sheep and pigs

Objectives: Study the diversity of temperate bacteriophages of 47 *P. multocida* isolates associated with different types of infection in cattle, sheep, pigs and poultry. The strains represented various capsular serotypes, outer membrane protein and multilocus sequence types.

Methods: Phage particles were induced with mitomycin C and characterized morphologically by transmission electron microscopy. The genetic divesity was studied using restriction endonuclease and Nucleotide sequence analysis of phage DNA.

Results: Both *Myoviridae* and *Siphoviridae* phages were identified in 29 isolates. Phage DNA was isolated from 18 isolates and 10 different RE types were identifed. Nucleotide sequence analysis of phage DNA from the same isolates demonstrated that both λ-phage and Mu-like phages were present in the same isolates of *P. multocida*. The results showed that more than one λ- phage and Mu-like phage were present in the majority of isolates. Sequence analysis of λ-phage genomes induced in toxigenic porcine strains of capsular types A and D demonstr5443ated the presence of the toxA gene. From our results we conclude that strains of *P. multocida* carry a diverse range of bacteriophages.These phages are likely to play important roles in the evolution and virulence of this important veterinary pathogen

PM384 New archaeal viruses discovered by metagenomic analysis of viral communities in enrichment culltures

David Prangishvili[1], Ying Liu[1], David Brandt[2], Sonoko Ishino[3], Yoshizumi Ishino[4], Eugene Koonin[5], Jörn Kalinowski[2], Mart Krupovic[6]

[1]*Institut Pasteur, Department of Microbiology, Paris, France*
[2]*Universität Bielefeld, Center for Biotechnology, Bielefeld, Germany*
[3]*Kyushu University, Department of Bioscience and Biotechnology, Fukuoka, Japan*
[4]*Kyushu University, Department of Bioscience and Biotechnology, Fukuoka, Japan*
[5]*National Institutes of Health, National Center for Biotechnology Information, Bethesda, United States*
[1]*Institut Pasteur, Department of Microbiology, Paris, France*

Background: Viruses infecting hyperthermophilic archaea thriving in extreme geothermal environments display enormous morphological and genetic diversity, and are classified into 12 families. Eight of these families include only one or two species, indicating sparse sampling of the crenarchaeal virus diversity

Objectives: . In an attempt to expand the crenarchaeal virome, we explored virus diversity in the acidic, hot spring Umi Jigoku in Beppu, Japan.

Methods: Environmental samples were used to establish enrichment cultures under conditions favoring virus replication. The host diversity in the enrichment cultures was restricted to members of the order *Sulfolobales*.

Results: Metagenomic sequencing of the viral communities yielded 7 complete or near-complete double-stranded DNA virus genomes. Six of these genomes could be attributed to polyhedral and filamentous viruses that were observed by electron microscopy in the enrichment cultures. Two icosahedral viruses represented species in the family *Portogloboviridae*. Among the filamentous viruses, two were identified as new species in the families *Rudiviridae* and *Lipothrixviridae*, whereas two other formed a group seemingly distinct from the known virus genera. No particle morphotype could be unequivocally assigned to the seventh viral genome, which apparently represents a new virus type. Our results suggest that filamentous viruses are globally distributed and are prevalent virus types in extreme geothermal environments.

PM385 Construction of replication-competent retroviral vectors expressing a fusogenic membrane glycoproteins

Yong-Tae Jung[1], Sae Young Jin[1], Eun Sik Lee[1], Byeng Kwon Kang[1], Seok Hwan Jung[1], Hang Bin Cho[1], Young Hyun Jeon[1]

[1]*Dankook University, Department of Microbiology, Cheonan-si, Korea, Rep. of South*

Background: Murine leukemia virus (MLV)-based replication-competent retrovirus (RCR) vectors developed as attractive tools for cancer therapy. RCR vectors have been shown to mediate efficient, selective, persistent tumor reduction in a wide variety of cancer model. Since the year 2000, fusogenic membrane glycoproteins(FMGs) have been applied to cancer therapy. FMGs are viral envelope proteins and kill cancer cells by fusing them into large multinucleated syncytia. In addition, the bystander effect of FMG killing is at least a log higher than that of suicide gene/prodrug systems such as herpes simplex virus thymidine kinase-1(HSVtk)/GCV or cytosine deaminase(CD)/5-fluorocytosine. Previous study report that an R peptide (16 amino acid cytoplasmic tail of transmembrane protein) truncated retroviral envelope protein can cause considerable cell-cell fusion.

Objectives: We have developed RCR vectors carrying FMGs for cancer gene therapy.

Methods: We generated replication-competent retroviral vectors based on Moloney MLV by replacing the native *env* gene in a full-length viral genome with R(-) envelopes of 10A1, 4070A, and GaLV.

Results: The resulting construct pCLXSN-10A1-R(-)-IRES-EGFP, pCLXSN-Ampho-R(-)-IRES-EGFP, and pCLXSN-GALV-R(-)-IRES-EGFP were able to replicate in 293T cells. In addition, syncytium formation was found in the 293T cells and HT1080 tumor cells after transfection of these vectors. To lyse tumor cells by syncytium and prodrug-activating transgene system, the plasmid pCLXSN-10A1-R(-)-IRES-yCD was generated by substituting the yeast CD gene into pCLXSN-10A1-R(-)-IRES-EGFP. The pCLXSN-10A1-R(-)-IRES-yCD showed increased specific activity in infected cells over infection with the pCLXSN-10A1-IRES-yCD. The combination of fusogenic oncolytic retroviral vectors with the CD/5FC strategy will be a useful tool for cancer gene therapy.

PM386 Modelling the sequence variation of Tick-borne encephalitis virus' non-structural proteins NS1, NS3 and NS5

Sarah Barrass[1]

[1]*University of Helsinki, Faculty of biological and environmental sciences, Helsinki, Finland*

Background: Tick-borne encephalitis virus (TBEV) is a pathogenic (+)ssRNA virus, within the flavivirus genus. Symptomatic infection with TBEV causes tick-borne encephalitis (TBE), a severe neurological disease, that causes long-term neurological deficits in up to 50 % of patients. Despite an available vaccine, the incidence of TBE has increased over 400 % during the last few decades, however, there are no specific antivirals available for the affected individuals. Structural and functional characterization of the TBEV virion and viral proteins could inform the identification of potential TBEV antivirals.

Objectives: The objective of the study is to investigate conservation of the TBEV non-structural proteins NS1, NS3 and NS5, and to map the sequence variation to structural models of the proteins. Thus we aim identify functional sites on those proteins that are conserved enough to be used in rational drug design.

Methods: The uniref90 and uniref50 databases were used to construct alignments to reveal the protein sequence diversity available in GenBank. The I-Tasser server was used to generate homology models for NS1, NS3 and NS5. The sequence variation was mapped onto the homology models and visualized using UCSF-Chimera.

Results: The predictive functional sites were shown to be more significantly conserved, with the majority of the sequence variation being located on the surface of the proteins. We can thus conclude that the homology models are of sufficient quality for the folds to be predicted correctly. Furthermore, the conserved functional sites can be used for rational drug design

PM387 Comparative genomic analysis of novel bacteriophages infecting vibrio parahaemolyticus isolated from western and southern coastal areas of korea

Jun-Hyeok Yu[1;2], Jeong-A Lim[1], Su-Jin Kwak[1], Jong-Hyun Park[2], Hyun-Joo Chang[1;3]

[1]Korea Food Research Institute, Food Safety Research Group, Wanju-gun, Korea, Rep. of South
[2]Gachon University, Department of Food & Biotechnology, Seongnam-si, Korea, Rep. of South
[3]Korea Research Institute of Chemical Technology (KRICT), Center for Convergent Research of Emerging Virus Infection, Daejeon-si, Korea, Rep. of South

Background: *Vibrio parahaemolyticus,* a harmful foodborne pathogen, has been facilitated to be resistant with antibiotics and now it is inevitable to find alternative bio-control agent like as bacteriophage.

Objectives: Six novel bacteriophages specific to *V. parahaemolyticus* (vB_VpaP_KF1~2, vB_VpaS_KF3~6) were characterized at molecular level. Our results could be used as reference to study phage genomics or apply of phages for future bio-control studies.

Methods: Genome of isolated *Vibrio parahaemolyticus* infecting phages were sequenced and annotated using Blastp based on e-value. Morphologic feature was examined with a transmission electron microscope. Also, there genomic and phylogenetic features were analysed using MEGA, ENDMEMO and others. Their genes were classified further into 5 clusters based on gene functions for structure, lysis, DNA packaging, DNA manipulation, and additional function.

Results: In this study, six KFs were classified into two groups based on genomic similarity. These two groups revealed different genomic features in size and G+C contents. They also belonged to different phylogenetic groups. In addition, each group had distinctive genes that the other group did not have, possibly causing different mechanisms at infection, assembly, and metabolism. Difference in morphological features between the two groups indicated that Group I belonged to *Podoviridae* while Group II belonged to *Siphoviridae*. In structure clusters, both groups had different structure proteins that could make phage infect bacteria efficiently. In addition, they had their own DNA packaging genes for virion assembly and lysis genes.

PM388 Comparative genomics of spontaneous Kayvirus host-range mutants

Roman Pantůček[1], Tibor Botka[1], Ivana Mašlaňová[1], Martin Benešík[1], Vladislava Růžičková[1], Jiří Doškař[1]

[1]*Masaryk University, Department of Experimental Biology, Faculty of Science, Brno, Czech Republic*

Background: Bacteriophages of the genus Kayvirus are valuable therapeutic agents against staphylococcal infections. Phage evolution is driven by emergence of resistance in their hosts and results in genomic heterogeneity by emerging recombination events and mutations in phage genomes.

Objectives: Spontaneous mutants of polyvalent bacteriophage 812 of the genus Kayvirus exhibiting a broader host range were isolated as rare plaques on various Staphylococcus aureus strains resistant to the parental phage. Here we describe results of the selection, lytic properties and comparative genomics of host-range mutants.

Methods: The lytic ability was tested on a set of 186 methicillin-resistant S. aureus (MRSA) representing the globally circulating clones. Deep sequence analysis of wild-type phage 812 and the most effective mutants was performed using the MiSEQ system. Variations in the phage genomes were analyzed using CLC Genomic Workbench.

Results: Five mutant phages were isolated as rare plaques on phage-resistant S. aureus strains. The host range of phage 812-derived mutants was 42% higher than the wild type. A considerable host-range extension was observed in phages containing a truncated endolysin gene and multiple changes in the host-takeover module. In addition, comparative genomics revealed that single-nucleotide polymorphisms from the parental phage 812 population were fixed in next-step mutants, mainly in the genes for structural proteins, receptor binding proteins, and the nucleic acid metabolism. We propose that the approach used for host-range mutant isolation is an effective and safe way to update the preparations against newly emerged phage-resistant strains.

Acknowledgement: The work was supported by grants 18-13064S and MUNI/A/0958/2018.

PM389 Characterization of human monoclonal antibody against Zika virus envelop protein using phage display

Hee-Jung Lee[1], Sehyun Kim[1;2], Hanul Choi[1;2], Young Bong Kim[1;2]

[1]Konkuk University, Biomedical science and engineering, Seoul, Korea, Rep. of South
[2]Konkuk University, Bio-industrial technologies, Seoul, Korea, Rep. of South

Background: Zika virus (ZIKV) is a mosquito-borne flavivirus which caused epidemic outbreaks and poses a significant threat to public health. Infection by ZIKV can be difficult to distinguish from infection by other flaviviruses due to high sequence similarity, antibody cross-reactivity, and virus co-circulation. ZIKV envelope (E) is an early surrogate marker of infection and can be used for the serological diagnosis of ZIKV infection.

Objectives: To develop a ZIKV-E specific monoclonal antibody, phage display method was used. The antibodies were selected through panning of the human synthetic Fab phage display library. Candidate antibodies were analyzed for affinity and stability against ZIKV, confirming the possibility of use as a diagnostic antibody.

Methods: The ZIKV-E antibodies were performed using a human synthetic Fab phage display library. Screened antibodies measured affinity by soluble ELISA, and analyzed thermal stability by protein thermal shift assay.

Results: Inactivated ZIKV was produced and biotinylated at a specific site for use as a target antigen during bio-panning. After four rounds of panning, a panel of 12 ZIKV-specific Fabs with various binding affinities was identified. Three high-binding clones (Z31B9, Z31G7, and Z32H10) were selected for further identification and converted into a full-length human IgG1 format. These anti-ZIKV IgGs were characterized for size, aggregation, and thermal stability. Binding activity of three IgGs with various ZIKV antigens was tested, it was confirmed that IgGZ32H10 had high affinity and no cross-reactivity. This anti-ZIKV IgG could be useful for the diagnosis of virus infection, a discovery of vaccine candidates, and evaluating vaccine potency.

PM390 Characteristics of wide-host-range lytic phages infecting multi-drug resistant Acinetobacter baumannii

Rapee Thummeepak[1], Thawatchai Kitti[2], Udomluk Leungtongkam[1], Slawomir Michniewski[3], Andrew Millard[4], Antonia P. Sagona[5], Sutthirat Sitthisak[1;6]

[1]*Department of Microbiology and Parasitology, Faculty of Medical Science, Naresuan University, Phitsanulok, Thailand*
[2]*Chiang Rai College, Chiang Rai, Thailand*
[3]*Warwick Medical School, University of Warwick, Coventry, UK*
[4]*Department of Genetics and Genome Biology, University of Leicester, United Kingdom*
[5]*School of Life Sciences, University of Warwick, Coventry, United Kingdom*
[6]*Centre of Excellence in Medical Biotechnology, Faculty of Medical Science, Naresuan University, Phitsanulok, Thailand*

Background: Multi-drug resistant *Acinetobacter baumannii* (MDRAB) is a major human pathogen, it is therefore necessary to find alternative treatments to tackle it.

Objectives: In this study, we characterized wide-host-range lytic phages infecting MDRAB.

Methods: In our previous work, we isolated five *A. baumannii* phages from waste water treatment plants and these phages were used in this study. Initially, spot tests for host range were performed by using 122 MDRAB isolates as hosts to identify broad-host-range phages. Wide-host-range lytic phages were selected to study morphological characteristics by transmission electron microscopy (TEM) and SDS-PAGE. Whole genome sequencing was conducted to analyze and identify genes encoding for lytic enzymes.

Results: Host range determination revealed that the percentage of infectivity by individual phages, ΦPAB02, ΦPAB04, ΦPAB19, ΦPAB29 and ΦPAB44 against tested MDRAB was 31.1, 25.4, 12.3, 19.7 and 20.5 %, respectively. We selected phages ΦPAB02 and ΦPAB04 that showed widest-host-range, for further charachterisation. TEM demonstrated that two phages could be classified as *Myoviridae*. Protein analysis revealed that there are differences in major protein bands between two phages. The results of genome analysis of ΦPAB02 and ΦPAB04 showed genome sizes to be approximatly 166 and 41 kb, respectively. Genome comparison with NCBI database showed that ΦPAB02 was most closely related to phage KARL-1, while ΦPAB04 was most closely related to phage phiAB06. Moreover, we identified putative genes encoding for tail-lysozyme and endolysin in the genomes of ΦPAB02 and ΦPAB04, respectively. Further work is needed to produce and characterize potential phage lytic enzymes to combat MDRAB.

PM391 Tapping into the metabolic cross-talk between a bloom-forming alga and its virus using mass spectrometry imaging

Guy Schleyer[1], Nir Shahaf[1], Carmit Ziv[2], Yonghui Dong[1], Roy A. Meoded[3], Eric J. N. Helfrich[3], Daniella Schatz[1], Shilo Rosenwasser[4], Ilana Rogachev[1], Asaph Aharoni[1], Joern Piel[3], Assaf Vardi[1]

[1]Weizmann Institute of Science, Department of Plant and Environmental Sciences, Rehovot, Israel
[2]The Volcani Center, Department of Postharvest Science of Fresh Produce, Institute of Postharvest and Food Sciences, Bet Dagan, Israel
[3]ETH Zurich, Institute of Microbiology, Zurich, Switzerland
[4]The Hebrew University of Jerusalem, Institute of Plant Sciences and Genetics in Agriculture, Rehovot, Israel

Background: Tapping into the metabolic cross-talk between a host and its virus can reveal unique strategies employed during infection. Viral infection is a dynamic process that generates an evolving metabolic landscape. Gaining a continuous view into the infection process is highly challenging and is limited by current metabolomics approaches, which typically measure the average of the entire population at various stages of infection.

Objectives: To study the metabolic basis of host-virus interactions between the bloom-forming alga *Emiliania huxleyi* and its specific virus.

Methods: We took a novel approach to study the metabolic basis of host-virus interactions by combining a classical method in virology, the plaque assay, with advanced mass spectrometry imaging (MSI), an approach we termed "*in plaque*-MSI".

Results: Taking advantage of the spatial characteristics of the plaque, we mapped the metabolic landscape induced during infection in a high spatiotemporal resolution, unfolding the infection process in a continuous manner. This landscape can be further linked to phenotypic plasticity induced during infection. Further unsupervised spatially-aware clustering, combined with known lipid biomarkers, revealed a systematic metabolic shift during infection towards lipids containing the odd-chain fatty acid pentadecanoic acid (C15:0), as well as a reduction in a potentially new class of lipids. Applying '*in plaque*-MSI' might facilitate the discovery of bioactive compounds that mediate the chemical arms race of host-virus interactions in diverse model systems.

PM392 Comparative genomic of Bowie and Cohen: two virulent Myoviridae suitable for coliform bacteria biocontrol

Felipe Molina[1], Alfredo Simancas[1], Rafael Tabla[2], Jose Emilio Rebollo[1], Isidro Roa[2], Antonia Gomez[2]

[1]*University of Extremadura, Genetics, Badajoz, Spain*
[2]*Scientific and Technological Research Centre of Extremadura (INTAEX – CICYTEX), Dairy Department, Technological Institute of Food and Agriculture, Badajoz, Spain*

Background: Coliform bacteria are considered a global health problem. They can be transmitted from person to person or by the ingestion of contaminated food or water. Additionally, coliforms might spoil processed food leading to alterations such as early blowing in cheesemaking. However, fermented dairy products cannot be treated antibiotics since they do not permit the growth of lactic bacteria which are important for the fermentation process. In this study, we characterized the genomes of two virulent *Myoviridae* bacteriophages (Bowie and Cohen) suitable for phage therapy against coliforms strains isolated from cheese samples and highly resistant to lab phages.

Objectives: Genome sequence analysis of Bowie and Cohen and comparative analysis with homologous coliphages to determine the potential use against coliforms.

Methods:

-Assembly of DNA sequences was achieved using MIRA.

- Open Reading Frame prediction was carried out with Prodigal and Prokka.

- Comparative genome analysis was carried out with several tools such as Mauve, CoreGenes and Phamerator.

Results: The double-stranded DNA genomes of Bowie and Cohen are composed of 50,821 bp and 169,066 bp with a G+C content of 44% and 40.5%, respectively. The genomes encode 83 and 262 putative ORFs, respectively. Comparative analysis revealed that there is a high degree of similarity between Bowie and pSf-1, whereas Cohen genome was highly similar to GEC-3S. Both phages depict extensive genomic mosaicism with low sequence overlapping. However, there was significant differences in the percentage of intergenic regions. Additionally, terminase DNA packaging enzymes and putative DNA methyltranferases were characterized.

PM393 Genome hypermobility by lateral transduction

Nuria Quiles[1], John Chen[2], Yin Ning Chiang[2], Rodrigo Bacigalupe[3], Alfred Fillol-Salom[1], Melissa Su Juan Chee[2], J. Ross Fitzgerald[3], José R. Penades[1]

[1]*University of Glasgow, Institute of Infection, Immunity and Inflammation, Glasgow, United Kingdom*
[2]*National University of Singapore, Department of Microbiology and Immunology, Singpaore, Singapore*
[3]*University of Edinburgh, The Roslin Institute, Edinburgh, United Kingdom*

Background: Bacteriophages play a crucial role in the genetic transfer between bacteria and phage transduction is generally regarded as the most important mechanism of horizontal gene transfer (HGT). Phage transduction is especially relevant as it allows bacteria to acquire virulence factors and antibiotic resistance genes.

Objectives: Using *Staphylococcus aureus* prophages as our model system, we aimed to characterise the role of integrated prophages in the transfer of bacterial genes after the induction of the lytic cycle by the bacterial SOS response.

Methods: In this study we have used a combination of classical molecular techniques such as allelic replacement and phage transduction experiments with different omics techniques like whole genome sequencing, RNA sequencing and transcriptome analysis.

Results: Our results suggest that *S. aureus* prophages have atypical lytic programs with delayed excision after the lytic cycle has been activated. The potentially detrimental effects of delayed excision are offset by *in situ* bidirectional replication, which creates multiple integrated phage genomes copies that are the substrate for both *in situ* DNA packaging and normal phage maturation that proceed in parallel. As a result of the *in situ* DNA packaging, large metameric spans, up to several hundred kilobases, of the *S. aureus* genome are packaged and transferred at very high frequency rates. Since bacterial chromosomes often contain multiple prophages, this new mechanism of bacterial transduction allows the transfer of a large portion of the bacterial genome at exceptionally high frequencies in a single lytic event.

PM394 Naturally occurring phages can effectively kill multi-drug resistant bacteria and show synergism thereby broadening its lytic host range

Roshan Nepal[1;2], Apsara Parajuli[2], Guna Raj Dhungana[2], Rajani Malla[2]

[1]*Center for Molecular Dynamics Nepal, Kathmandu, Nepal*
[2]*Central Department of Biotechnology, Tribhuvan University, Kirtipur, Nepal*

Background: Antimicrobial resistance (AMR) is no more an impending threat but real global health crisis. Without effective alternatives, infections once easily treatable will again kill. Phage therapy, using virus to kill bacteria, is gaining traction as it has shown promising results lately and is seen as last-resort experimental approach when all available antibiotics fail.

Objectives: This study aims to isolate lytic phages against drug-resistant human pathogens, study its lytic efficiency in varying temperatures, pHs, mouse models and analyze its genome.

Methods: Phages were isolated employing double layer agar assay from river water (25) as 'phage source' using 120 bacterial strains (representing 10 genera) as 'hosts'. Phages were purified and assayed for multiple host range (MHR) by spot assay. Lytic efficacy on different stages of bacterial life-cycle, different temperature and pH was analyzed using broth-assay and spectrophotometry. Potent phages were identified by electron microscopy and finally, genome sequencing of phages was done to study potent lysin enzymes.

Results: One hundred sixteen naturally occurring lytic phages were isolated that could kill drug-resistant bacteria. Among them, 109 (93.9%) showed MHR within genus whereas mixture of phages lysed strains that individual phages couldn't, suggesting synergistic lytic mechanism. Phages were significantly efficient in killing bacteria in lag, log and stationary phase, were stable around human body temperature and blood pH. TEM confirmed them as Caudovirales. Whole genome sequencing identified specific 'lysin' enzyme that was conserved and could serve a broad range dynamic 'antibiotics' that could kill specific strains leaving beneficial microbiome unharmed.

PM395 Double combinations applied via course of consecutive alternating administration in Coxsackievirus B3 infected mice

Adelina Stoyanova[1], Stefan Philipov[2], Gerhard Pürstinger[3], Vadim Makarov[4], Angel Galabov[1]

[1]*The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, Virology, Sofia, Bulgaria*
[2]*Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Sofia, Bulgaria*
[3]*Institute of Pharmacy, University of Innsbruck, Innsbruck, Austria*
[4]*Bach Institute of Biochemistry, Moscow, Russian Federation*

Background: Our previous studies ascertain the efficacy of consecutive alternating administration (CAA) of two triple combinations: pleconaril /guanidine/oxoglaucine (PGO) and pleconaril/MDL-860/ oxoglaucine (PMO) against Coxsackievirus B1 (CVB1) infection in newborn mice. It was shown that these drug combinations prevented the development of drug resistance in virus progeny.

Objectives: In the present study, we test the effect of the double combinations of the same compounds in experimental Coxsackievirus B3 (Woodruff strain) infection in newborn mice infected subcutaneously with 20 MLD_{50}.

Methods: Cumulative mortality (percentage), mean survival time (MST) (days) and weight (in grams) of suckling mice were recorded.

Results: The obtain results indicate efficacy of PG, PO and PM combinations administered according to the CAA treatment schedule in CVB3 infected mice - decreased mortality rate and lengthening of the mean survival time (MST). In comparison with placebo groups the monotherapeutic course with pleconaril demonstrated some independent antiviral effect. It was found that oxoglaucine, MDL-860 and guanidine.HCl monotherapies were without a marked antiviral effect.

PM396 Effect in vitro of double combinations against Coxsackievirus B3

Adelina Stoyanova[1;2], Luchia Mukova[2], Stefan Philipov [3], Gerhard Pürstinger [4], Vadim Makarov[5], Angel Galabov[2]

[1]*The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, Virology, Sofia, Bulgaria*
[2]*The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, Sofia, Bulgaria*
[3]*Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Sofia, Bulgaria*
[4]*Institute of Pharmacy, University of Innsbruck, Innsbruck, Austria*
[5]*Bach Institute of Biochemistry, Moscow, Russian Federation*

Background: Coxsackievirus B3 (CVB) is one of the major causative pathogens of viral myocarditis. Although most CVB-related cardiac illnesses are subclinical, severe viral myocarditis can lead to heart failure or sudden cardiac death. There are no enterovirus-specific drugs available for clinical use because of fast development of drug-resistant and even drug-dependent mutants. Synergistic combinations of two or more agents can overcome toxicity and other side effects associated with high doses of single drugs, by either countering biological compensation, sparing doses on each compound, or accessing context-specific multi-target mechanisms, also may restrict the emergence of resistance to the partners in the combination.

Objectives: We studied the combined effects in cell culture of inhibitors with different mode of action against cardiotropic Coxsackievirus B3 (Woodruff strain).

Methods: Theoretical additive interactions of expected effects for drug–drug interactions was calculated by using MacSynergy II (Prichard et al.,1993). Interpretation of significance of the observed volumes of synergy or antagonism, depicted in the differential surface plots, was based upon the program guidelines.

Results: An additive effect was exhibited when pleconaril and guanidine hydrochloride were combined with oxoglaucine. The combinations of pleconaril with guanidine and MDL-860 manifested moderate synergy, while combinations of MDL-860 with oxoglaucine and guanidine revealed strong synergy. Combinations of pleconaril, MDL-860, oxoglaucine and guanidine hydrochloride had no cytotoxicity effect on uninfected HEp-2 cells.

Acknowledgements: Part of this work is financially supported by National Science Fund of Bulgaria, Project ДН 11/12/2017.

PM397 Human Herpes Virus Infections in Bulgarian Patients with Myalgic Encephalomyelitis/Chronic Fatigue Syndrome

Evelina Shikova[1], Valentina Reshkova[2], Antoniya Kumanova[1], Sevdalina Raleva[1], Dora Alexandrova[3]

[1]*National Center of Infectious and Parasitic Diseases, Virology, Sofia, Bulgaria*
[2]*University Hospital "St Ivan Rilski", Sofia, Bulgaria*
[3]*The National Specialized Hospital for Active Treatment in Haematological Diseases, Sofia, Bulgaria*

Background: Infections of cytomegalovirus (CMV), Epstein-Barr virus (EBV) and human herpesvirus-6 (HHV-6) are suspected as etiological agents for Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS). It was suggested that persistent infection with these viruses could trigger a chronic immune activation of cytokines leading to ME/CFS.

Objectives: To determine frequency of EBV, CMV and HHV-6 active/persistent infection in Bulgarian patients with ME/CFS.

Methods: Blood samples from ME/CFS patients (n=48) and healthy controls (n=40) were included in the study. DNA from plasma and peripheral blood mononuclear cells (PBMCs) was tested for CMV, EBV and HHV-6 by PCR. Criteria for persistent and active viral infection were presence of viral DNA, respectively, in PBMCs and plasma samples. Statistical analysis was performed using Fisher exact test ($p < 0.05$ considered statistically significant).

Results: In ME/CFS plasma samples, EBV DNA was found in 16/48 (33.3%), CMV DNA – in 3/48 (6.3%) and HHV-6 DNA in 1/48 (2%) of samples. EBV DNA was detected in 2/40 (5%), CMV and HHV-6 DNA were not found in plasma samples of controls. The difference in frequency of EBV active infection in ME/CFS and control group was statistically significant ($p=0.0011$), suggesting a possible role of EBV in ME/CFS pathogenesis. There was no statistically significant difference for CMV and HHV-6. The frequency of viral genome detection in PBMCs of patients and controls was 81% vs 80% for EBV and HHV-6 and 75% vs 80% for CMV, indicating high but similar frequency rates of persistent infections with these viruses among ME/CFS patients and healthy persons.

PM398 Switching alternative in vitro poteny test method for Japanese encephalitis vaccine quality control

JiYoung Hong[1], Won Gyo Jang[1], Dawoon Jeong[1], Naery Lee[1], Sang Ja Ban[1]

[1]*National Institute of food and drug safety evaluation, Vaccine division*

Background: Potency testing is an essential component of Japanese encephalitis virus(JEV) vaccine quality control. Traditionally, the animal-based assay (*in vivo* potency test) for the quality control for JEV vaccines has been used as a "gold standard method" for more than 50 years. However, the animal-based assay has several problems, among which the most serious problem is inherent variability and ethical issues. Recently, the global trend in routine control of vaccines has been based on the 3R principle and monitoring the consistency of vaccine.

Objectives: This study was conducted with the aim of establishing the non animal test to replace the *in vivo* potency test of JEV vaccine, thus measuring the JEV antigen quantitatively and ensuring its validity in representing the immunological functionality of the JEV antigen.

Methods: We developed a double-sandwich enzyme-linked immunosorbent assay (DS-ELISA) that could detect JEV envelope (E). There are two Ab used in DS-ELISA, a neutralizing mAb for the JEV E protein. Using DS-ELISA method, a test method for quantitatively measuring the antigen of the vaccine was conducted to verify linearity, accuracy, and specificity, and a comparison *in vivo* and *in vitro* test was conducted using a reduced-potency samples exposed to a high temperature to determine the correlation or relationship between the two test methods.

Results: As a result of conducting the method validation, the linearity, accuracy, and specificity were satisfied. In addition, the results of a comparative test for reduced-potency samples were compared with the results of the relative potency between the two test methods.

PM399 The role of mobile genetic elements in porcine enterotoxigenic E.coli virulence and ecology.

Michela Gambino[1], Lone Brøndsted[1]

[1]*University of Copenhagen, Department of Veterinary and Animal Sciences , Frederiksberg, Denmark*

Background: Enterotoxigenic *Escherichia coli* (ETEC) are the main cause of diarrhea in post-weaning piglets. They are characterized and classified according to the presence of two virulence factors (fimbriae and enterotoxins), usually encoded by plasmids or prophages. Despite the importance of mobile genetic elements (MGE) in ETEC virulence, no report explains the role of the chromosomal background in accepting MGE, nor the contribution of MGE in ETEC diversity and sensitivity to their natural predators, the bacteriophages.

Objectives: We aim to explore ETEC chromosomal background for MGE acceptance and the contribute of MGE in ETEC diversity and sensitivity to bacteriophages.

Methods: We gathered a collection of 110 ETEC strains isolated from piglets in Europe, affected by post-weaning diarrhea. We characterized the fimbriae and toxin types and investigated their genetic diversity by Pulse Field Genetic Electrophoresis. The presence of MGE has been explored by plasmid profile analysis and mitomycin induction of prophages. We hypothesize that the high number of prophages in the ETEC genomes could be responsible for the immunity against other bacteriophages, since it has been much easier to isolate temperate than lytic bacteriophages against these ETEC strains. Interestingly, the genome analysis of a lytic T7-like bacteriophage revealed a known anti-resistance mechanism against restriction-modification enzymes that could explain its high infectivity against ETEC.

Results: Our 110 ETEC are diverse in the virulence factors and in the genetic background. MGEs could be responsible for the ETEC resistance to previously isolated bacteriophages. Nevertheless, these can be overcome by ETEC-specific bacteriophages.

PM400 Structure of the HSV-1 Portal and Portal Vertex-Associated Tegument by High-Resolution CryoEM Focussed Classification Reconstruction

Angus Lovely[1], Swetha Vijayakrishnan[1], David Bhella[1]

[1]*MRC-University of Glasgow Centre for Virus Research, Glasgow, United Kingdom*

Background: Herpes simplex virus 1 (HSV-1) is a common viral infection, estimated to infect more than 67% of the global population. Infection results in painful blistering or ulceration ('cold sores' and genital herpes) and can also cause serious illness, including fatal encephalitis. HSV-1 infections are lifelong due to latent infections of nerve cells that periodically reactivate, and there is no cure.

HSV-1 virions contain a proteinaceous tegument layer and icosahedral capsid, one vertex of which is the portal structure. The portal is essential in the viral life cycle as it nucleates capsid assembly, is the site of DNA-packaging and -release, and interacts with the nuclear pore complex during infection. The portal vertex-associated tegument (PVAT) is a 5-helix heteropentameric bundle that connects the portal and tegument. The PVAT is thought to consist of pU_L17, pU_L25 and putatively pU_L36 (large tegument protein).

Objectives: To achieve a high-resolution description of the HSV-1 capsid, reconstructed to reveal the portal and PVAT from wild type (WT) and pU_L36-deficient virus.

Methods: HSV-1 WT and mutant capsids, purified from nuclear extracts, were imaged on a JEOL CryoARM-300 transmission electron microscope. Focussed classification, a novel technique, was used for 3D reconstruction. This allows features that do not fit icosahedral symmetry, such as the portal and PVAT, to be reconstructed to a high resolution.

Results: Using our high-resolution reconstruction, we have unambiguously shown morphological differences in the PVAT between WT and pU_L36-deficient capsids. This challenges the current understanding in the field that pU_L36 is a subunit of the PVAT.

PM401 Monolith chromatography - new possibilities in virus separation and purification

Mladen Krajacic[1]

[1]*University of Zagreb, Department of Biology, Zagreb, Croatia*

Background: The structure of monolithic chromatographic supports, makes them suitable for separation and purification of virus particles.

Objectives: Ion-exchange chromatography is expected to separate mixed viruses as long as they have different charge properties. In addition, viruses might be engineered in order to shift their particles from interfering material. We could improve viral chromatographic "separability", abolishing neither its structural stability, nor its biological activity.

Methods: The results revealed from HPLC using strong anion exchanger on methacrylate monolithic columns. Following short low-speed centrifugation and filtration, tissue homogenate or a cell lysate was loaded to the short monolithic disc and eluted with a gradient of loading/eluting buffer. The flow rate was 2 mL/min.

Results: Plant viruses, with different isoelectric point, morphology and construction type, were separated from one another in a single chromatographic experiment from an artificially mixed sample. Two strains of one of them were separated, which makes four virus peaks in total, with the baseline separation of the distinctive strains. At the same time, the virus recovery was very high, up to 95%. By using absorption ratio at different wavelengths, 260 and 280 nm, it was possible to attribute specific peak to icosahedral or helical structure.

Adenoviral vectors, rearranged in the main structural proteins, were analysed to quantify changes required for modification of viral chromatographic features. The modifications have been created earlier with the purposes related to gene therapy. A clear chromatographic shift was obtained for the recombinant that missed just two negatively charged amino acids in the hexon protein.

PM402 A novel cytomegalovirus vaccine encoding the pentameric complex completely protects against congenital CMV

Alistair McGregor[1], K. Yeon Choi[1]

[1]*Texas A&M Health Science Center, Bryan, United States*

Background: Congenital cytomegalovirus (cCMV) is a leading cause of deafness and mental retardation in newborns and there is no vaccine. The guinea pig is the only small animal model for cCMV but requires guinea pig CMV (GPCMV). Neutralizing antibodies are likely important for protection against cCMV. Human CMV and GPCMV encode a glycoprotein pentamer complex (PC) for infection of non-fibroblast cells.

Objectives: Evaluation of efficacy of CMV vaccine encoding viral pentamer complex against cCMV

Methods: A GPCMV BAC was modified to generate a disabled infectious single cycle (DISC) vaccine that lacks an essential capsid gene. Two DISC strains were generated: DISC-I, PC-negative; DISC-II, PC-positive.

Results: Vaccines induced antibodies to viral glycoprotein complexes (gB, gH/gL/gO, and gM/gN). Additionally, DISC-II induced antibodies to PC. DISC-II neutralization titers on fibroblast, epithelial and trophoblast cells were significantly higher but both vaccines induced similar T-cell response. In congenital protection studies, dams in two groups were vaccinated and mated: DISC-I(N=14); DISC-II(N=19). Group 3 included as non-vaccinated (N=15). In 2nd trimester, animals were challenged with wild-type GPCMV (10^5 pfu). Pup outcome and viral load in target organs were analyzed. Live pup births in DISC groups (94.1-96.8%) were significantly higher than non-vaccinated (56.3%) suggesting both vaccines were successful. However, cCMV rate was significantly reduced in DISC-II pups (0% detectable virus) in pup organs, compared to non-vaccinated group (80%) and DISC-I pups (36%). Overall, PC+ DISC vaccine had better neutralizing antibodies and protection against cCMV. Based on these results a human CMV DISC vaccine would be similarly effective.

PM403 Infectious pancreatic necrosis virus modulates epigenetically the anti-viral response in salmonid cells

Juan Guillermo Cárcamo[1;2], René A. Manríquez[1;2]

[1]*Interdisciplinary Center for Aquaculture Research (INCAR), Biochemistry and Microbiology, Vald, Chile*
[2]*Institute, Universidad Austral de Chile, Valdivia, Chile*

Background: The infectious pancreatic necrosis virus (IPNV) is the viral pathogen with the highest dissemination in salmonids, generating considerable economic losses in the salmon industry. The main mechanism of response to viral infection is the activation of the interferon system (IFN). The mechanism by which IPNV interacts with the IFN system has not yet been elucidated

Objectives: Using RTS-11 cells (rainbow trout monocyte/macrophage), we studied the effect of IPNV infection on epigenetic mechanisms associated with two antiviral response cytokines (IFN1 and IFNγ2), and the enzymes DNA methyl transferase (DNMT), Histone acetyl transferase (HAT) and Histone deacetylase (HDAC).

Methods: These studies show that only IFNγ2 has CpG islands in its promoter, and that these are methylated during infection with IPNV. This promoter hypermethylation is consistent with increases in DNMT expression. Furthermore, during the infection an increase in the acetylation of H4 histones is observed in the DNA regions associated with the promoters of both cytokines (IFN1 and IFNγ2). This effect coincides with increases in HAT expression, as well as the subsequent decrease in acetylation of histone H4, correlated with increases in HDAC expression.

Results: Taken together, we present evidence that during IPNV infection, different cellular mechanisms of epigenetic regulation are modulated and that this could be associated with the disturbance of the innate defense observed in salmonids infected with IPNV.

Funding: CONICYT – CHILE grants, FONDECYT 1150934 & FONDAP 15110027.

PM404 Absence of major integrase strand transfer inhibitors resistance mutation in treatment naive hiv 1 infected patients in the eastern cape, south africa

Tennison Digban[1;2], benson c iweriebor[2], larry c obi[2], uchechukwu nwodo[1;2], anthony i okoh[1;2]

[1]*university of fort hare, applied environmental and microbiology research group, Alice, South Africa*
[2]*university of fort hare, biochemistry and microbiology, Alice, South Africa*

Background: The evolution of drug resistance is a salient threat in the extensive management of HIV-1 infected individuals. Rising episodes of confirmed resistance to first and second line antiretroviral (ARV) therapy have impelled the South African national treatment programme and many other countries to introduce third-line treatment option with integrase inhibitor as a component.

Objectives: This study is aimed to analyse the entire HIV-1 integrase gene from patients not on ARVs in selected clinics within the Eastern Cape, South Africa

Methods: Viral RNA was extracted from blood samples of 60 diagnosed HIV-1 patients attending some HIV testing and counselling clinics from July 2016 to May 2017. Integrase gene was amplified with specific primers by RT-PCR and nested PCR. The amplified products were sequenced using the ABI 316 sequencer, edited and translated into amino acid with Geneious version 9.1.5., while multiple amino acid alignment was done using BioEdit. Integrase sequences were submitted to Stanford HIV drug interpretation programme for query of drug resistance associated mutations while phylogenetic analysis was performed using MEGA 7 to delineate all viral sequences into subtypes.

Results: From the study, a total of 51/60 (85.0%) reliable HIV-1 integrase sequences was obtained with subtyping and drug resistance mutations performed. No major resistance associated mutation to the integrase sequence was observed. Only 1(1.9) case of polymorphic accessory E157Q mutation was observed. Another minor mutation (L74M/L) which is a highly polymorphic accessory mutation was identified in 2(2.9%) viral sequence. Phylogenetic analyses delineated all the sequences as HIV-1 subtype C.

PM405 Identification of a quaternary ammonium compound with antiviral activity against herpes simplex viruses type 1 and type 2 in human gingival fibroblast

Diana Alvarez[1], Luisa Duarte[1], Patricio Smith[2], Pablo González[1]

[1]Millennium Institute on Immunology and Immunotherapy, Pontificia Universidad Católica de Chile, Departamento de Genética Molecular y Microbiología, Facultad Ciencias Biológicas, Santiago, Chile
[2]Pontificia Universidad Católica de Chile, Escuela de Odontología, Facultad de Medicina, Pontificia Universidad Católica de Chile., Santiago, Chile

Background: Herpes simplex viruses (HSV-1 & HSV-2) elicit numerous clinical manifestations in humans, such as skin lesions in the orofacial area and genitalia, as well as herpetic gingivostomatitis. Acyclovir, a nucleoside analogue commonly used to treat HSV infection is poorly effective for treating herpetic skin lesions when applied as a cream. Furthermore, acyclovir-resistant isolates have been isolated from immunosuppressed patients, thus requiring therapeutic alternatives. Cetylpyridinium chloride (CPC) is a quaternary ammonium compound with bactericidal properties that is frequently added to mouthwashes and deodorants; nevertheless it was recently reported to have virucidal activity against influenza A virus.

Objectives: We sought to assess the potential antiviral effects of CPC against HSVs.

Methods: Epithelial cells and human gingival fibroblasts were infected with HSV-1 or HSV-2 strains that express a green fluorescent protein (GFP) and then treated with CPC. Infection and viral processes were then followed within the infected cells.

Results: We found that cells infected with HSV and then treated with CPC produced significantly less virus plaque forming units and displayed reduced expression of virus-encoded genes as compared to controls. Dissection of the viral step inhibited by CPC treatment indicates that CPC inhibits the transcription of viral gene early after infection by blocking the translocation of NF-κB to the nucleus of HSV-infected cells. Taken together, our results suggest that CPC has anti-HSV effects which is mediated by non-virucidal effects, but rather the modulation of cellular signalling events required by HSVs for replication.

PM406 Bacterial as anticancer agents

Chung-Jen Chiang[1], Yun Peng Chao[2], Po-Han Huang[3]

[1]China Medical University, Department of Medical Laboratory Science and Biotechnology, Taichung, Taiwan
[2]Feng Chia University, Department of Chemical Engineering, Taichung, Taiwan
[1]China Medical University, Department of Medical Laboratory Science and Biotechnology, Taichung, Taiwan

Background: In Taiwan, cancer are the most deadly disease, according to the statistics of the Ministry of Health and Welfare in 2015, colorectal cancer has ranked first in probability of having cancer for 10 consecutive years. In fact cancers are seriously problems in many countries but traditional chemotherapy and radiotherapy will damage normal cell and decline immunity. Using bacteria as anti-tumor agents have many advantages such as low-cost, easy to make genetic engineering and tumor colonization.

Objectives: In this study, we used probiotics *E. Nissile* as potential anti-tumor agents. Therefore, first, we generated hemolysin as antitumor agent for cancer therapy.

Methods: We use probiotics E. Nissile which generated affibody and hemolysin as a new anti-tumor agent to target and kill EGFR overexpression tumor cell.

Results: Our results showed that : (1)Under microscope, through trypan blue staining, the SW620 and HT29 cell lines were exposure with hemolysin expressed from E. coli for 15 minutes and 30 minutes, respectively, the cells surface were formation of blisters. The cell injury and death is accompanied by cell swelling. (2) Next, MTT assay, we found that hemolysin E that generated by E. Nissile can could cause cytotoxicity of cancer cell, and after incubation for 3 hours, the half survival rate (IC50) of HT29 cells were 2.36 g/ml and SW480 cells were 0.84 g/ml, (3) In vivo test, treatment with hemolysin-expression E. coli were found within HT-29 tumor and the size of tumor was 59.31% smaller than control mice.

PM407 Isolation and characterization of agar-degrading bacteria from a marine ecosystem

Ashraf Khalifa[1]

[1]*King Faisal University, Biological Science, Saudi Arabia*

Background: m Agar is a mixture of heterogeneous galactans and is a major component of the cell walls of certain algae. It has and many industrial applications, due to its jellifying properties. Many microbes consume agar as a carbon and energy sources.

Objectives: The aim of the current study was to isolate and characterize agar-degrading bacteria from the marine ecosystem in the Al-Ahsa region, Saudi Arabia.

Methods: Water samples were collected from three different points in the Arabian Gulf. Three strains were obtained from water samples after enrichment with agar as a sole energy and carbon source. The strains were mainly characterized based on their physiological and biochemical features using the Biolog Gen III MicroPlate test system in addition to 16S rRNA gene sequencing.

Results: The strains were diverse with respect to phenotypic characteristics including antibiotic resistance. ADB-1, ADB-2 and ADB1-3 reacted positively to 45 (~48%), 82 (~87%) and 16 (17%), respectively, of the 94 diverse characteristics investigated. Additionally, all strains exhibited good growth at 10 % NaCl. The strains were clustered within the genus *Pseudoalteromonas* as revealed by the results of the comparative sequence analysis of the 16S rRNA gene at identity levels more than 99.5%. These results indicated that marine ecosystems in Al-Ahsa harbor agar-degrading bacteria, which could be exploited for potential biotechnological applications.

PM408 Sustained release zinc chitosan nanoparticles and zinc solubilizing bacteria for growth promotion in capsicum annuum l

Gauri Achari[1], meenal kowshik[1]

[1]*Birla Institute of Technology and Science Pilani KK Birla Goa Campus, Zuarinagar, India*

Background: Plant growth promoting bacteria (PGB) as biofertilizers are ecofriendly means for increasing plant productivity. Studies have reported the positive effects of micronutrient nanoparticles (NP) mainly Zinc (Zn) on the plant growth.

Objectives: The current study investigates the effect of Zinc oxide (ZnO) NP and sustained release Zn-chitosan NP on Zn solubilizing PGB strains *Bacillus amyloliquefaciens* XB153 and *Pseudomonas aeruginosa* XB7.

Methods: Zn solubilization by XB153 and XB7 was tested in medium with ZnO. ZnO-NP were purchased from Sigma Aldrich (USA) whereas the sustained release Zn-Chitosan NP were synthesized using $ZnSO_4$ and chitosan. NP were characterized by Scanning electron microscopy (SEM), X-ray diffraction (XRD) and Fourier Transform Infra-Red spectroscopy. NP were tested for growth enhancement in *Capsicum annuum* seedlings and their minimum inhibitory concentration (MIC) on XB153 and XB7 was determined by measuring growth in medium with NP.

Results: XB153 and XB7 had Zn solubilizing efficiency of 160 and 500 respectively. Zn-chitosan NP were nanospheres (diameter 31.41 nm). XRD and FT-IR indicated Zn complexation in chitosan. NP (100-200 $\mu g.g^{-1}$) increased growth and biomass in *C. annuum*. MIC of Zn-Chitosan NP in XB153 and XB7 was 1000.0 $\mu g.L^{-1}$. The MIC of ZnO-NP was 60.0 $\mu g.L^{-1}$ for XB153 and the growth of XB7 was unaffected in presence of ZnO-NP at a concentration of 8000.0 $\mu g.L^{-1}$. The results indicate that Zn-Chitosan NP at a concentration suitable for plant growth enhancement are non-deleterious to PGB.

PT001 Petroleum hydrocarbon-biomarkers from prokaryotes biomass

Aleksandra Poshibaeva[1], Alina Gayanova[1], Maxim Giruts[1], Anna Perevalova[2], Ekaterina Semenova[2], Guram Gordadze[1]

[1]Gubkin Russian State University of Oil and Gas (National Research University), Moscow, Russian Federation
[2]Winogradsky Institute of Microbiology (Biotechnology Research Centre), Russian Academy of Sciences, Moscow, Russian Federation

Background: According to the organic theory of petroleum generation, it is assumed that hydrocarbon- biomarkers originated from the remains of living organisms that inhabited the Earth during the early geological periods. Current research in petroleum origin focuses on eukaryotic organisms, whereas prokaryotes are considered as applied to the transformation of eukaryotic organic matter. However, we have shown recently that petroleum biomarkers can be obtained from the prokaryotes biomass.

Objectives: Research of the biomarkers in the soluble part (products of life) and thermolysis products of the insoluble part (kerogen) of the archaea *Thermoplasma* sp. and bacteria *Arthrobacter* sp., *Pseudomonas aeruginosa*, *Geobacillus jurassicus, Shewanella putrifaciens*.

Methods: The prokaryotes were isolated and caltured. The soluble part of the prokaryote liophylic biomass was extracted completely. The insoluble part of the biomass was subjected to termolysis at 330 ^{o}C in a sealed ampoule. Hydrocarbons in the soluble and insoluble parts were analyzed by GC and GC-MS. In additional, Rock-Eval pyrolysis of the biomass was conducted.

Results: Petroleum biomarkers (*n*-alkanes, isoprenanes, pregnanes, steranes, cheilanthanes, hopanes) in soluble part and thermolysis products of the insoluble part of the biomass of archaea have been identified. It should be noted that all of these biomarkers are found in the thermolysis products of the insoluble part of all bacteria. Only *n*-alkanes and isoprenanes have been identified in the soluble part. The distribution of biomarkers resembles marine organic matter, a fact that is confirmed by Rock-Eval pyrolysis data. Squalene has been found in all samples.

PT002 Biosynthesis of silver nanoparticles mediated by aqueous extract of thuja orientalis leaf: characterization and potential application

Kunle Okaiyeto[1], Leonard Mabinya[1], Uchechukwu Nwodo[1], Anthony Okoh[1]

[1]*University of Fort Hare, Biochemistry and Microbiology, Alice, South Africa*

Background: The synthesis of metal nanoparticles using biological methods has gained biotechnological attention due to their cost-effectiveness, eco-friendly, less toxicity and potential applications in nanomedicines.

Objectives: In this present study, aqueous extract of *Thuja orientalis* leaf was used as a bioreducing agent to synthesize silver nanoparticles (SNPs), characterization and the biological activities of the SNPs were investigated.

Methods: The bio-reduction potential of the aqueous extract was confirmed by monitoring the colour change using UV–Visible spectroscopy. The synthesized silver nanoparticles were further characterized with transmission electron microscopy (TEM), X-ray diffraction (XRD), scanning electron microscopy (SEM), Energy dispersive x-ray spectroscopy (EDX) and Fourier transform infrared spectroscopy (FTIR). The cytotoxicity potential of the synthesized silver nanoparticles was assessed against HeLa cells and other biological activities such as antimalarial, antitrypanosomal, antibacterial (against both gram-negative and gram-positive bacteria) and antioxidant activities of SNPs were investigated afterwards.

Results: The synthesized nanoparticles showed a strong peak at 420 nm under UV-Vis and TEM results showed that the particles were spherical with size ranging from 25 to 46 nm. The XRD pattern showed the crystalline structure with face-centered cubic geometry. EDX analysis revealed that the particles predominantly composed of carbon, oxygen and silver and FTIR revealed the key phytochemicals, which serve as the reducing and stabilizing agents. The results from the biological activities showed that the synthesized nanoparticles has no cytotoxic effect against HeLa cells and had strong antimalarial (IC_{50} = 2.9), antitrypanosomal (IC_{50} = 5.3), antibacterial and antioxidant agent which could be useful in medicine.

PT003 Effects of Jatropha curcas leaves extract on biochemical parameters of Plasmodium berghei infected mice

Chiedu Edomaodu[1,2]

[1]*Department of Biochemistry and Microbiology, Faculty of Science and Agriculture, University of Fort Hare, Alice, South Africa*
[2]*Department of Biochemistry, School of Natural, Sciences Faculty of Sciences, Joseph Ayo Babalola University, Ikeji-Arakeji, Nigeria*

Background: Malaria is one of the most global health concerns and is been considered as one of the world deadly diseases. Infant and pregnant woman are most vulnerable to this disease and it has necessitated the need for discovery of alternative antimicrobial agents to tackle these challenge.

Objective: To ascertain serum enzyme level on alanine aminotransaminase (ALT), aspartate aminotransaminase (AST), alkaline phosphatase (ALP), of the mice under Plasmodium condition treated with *Jatropha curcas*.

Methods: Forty-eight albino mice of weight below 25g each were divided into six study groups of eight mice in each study group. Forty mice were infected with *Plasmodium berghei* and the remaining eight mice serve as the positive control group. Histology of Liver was carried out. Parasitized mice receive 400mg/kg, 200mg/kg, and 100mg/kg of ethanolic extract of *J. curcas* leaf.

Phytochemical screening of *Jatropha curcas* leaf indicates the presence of cardiac glycoside, tannin, flavonoid, alkaloid, phylobatanin and anthraquinone.

Results: Body weight of all group mice was reduced, serum enzyme of mice was altered. Histology of liver reveals some damage. *J. curcas* leaf possesses significant anti-plasmodial.

PT004 Identification of genes associated with production of selenium nanoparticles in Pseudomonas putida KT2440

Roberto Avendaño[1], Sofía Vieto[1], Rafael Montenegro[1], Nicole Ramírez[2;3], Rufus Frew[4], Manuel Salvador[4], Juhyun Kim[4], José I. Jiménez[4], Max Chavarría[1;2;3]

[1]*Centro Nacional de Innovaciones Biotecnológicas (CENIBiot), CeNAT-CONARE, San José, Costa Rica*
[2]*Universidad de Costa Rica, Escuela de Química, San José, Costa Rica*
[3]*Universidad de Costa Rica, Centro de Investigaciones en Productos Naturales (CIPRONA), San José, Costa Rica*
[4]*University of Surrey, Faculty of Health and Medical Sciences, Guildford, United Kingdom*

Background: Selenium (Se) is an essential element for the cell that has multiple applications in medicine and technology; microorganisms play an important role in Se transformations in the environment. Recently, we reported the ability of the soil bacterium *Pseudomonas putida* KT2440 to synthesize nanoparticles (range 70-360 nm) of elemental selenium (nano-Se) from selenite (Avendaño et al. 2016), however, the genes or enzymes involved in the process remain unknown.

Objectives: Identify genes associated to nano-Se biosynthesis in *P. putida* KT2440

Methods: The mutagenesis strategy for identifying genes associated to nano-Se production was performed with the pBAM1 vector (Martínez-García et al. 2011). Strains were cultured in LB medium supplied with selenite (1 mM). Bacterial growth was estimated by monitoring the OD_{600} in a Synergy H1 Hybrid Multi-Mode Reader at 30 °C.

Results: Twelve mutants related to selenite reduction were isolated. Mutants unable to carry out the reduction process were affected in genes that encode enzymes involved in sulfur metabolism (e.g. PP_2371 encoding sulphite reductase hemoprotein; PP_3999 encoding uroporphyrin-III C-methyltransferase) as well as redox regulation (e.g. PP_3998 encoding glutathione S-transferase domain-containing protein; PP_4189 encoding 2-oxoglutarate dehydrogenase). In addition a mutant capable of reducing selenite at a higher rate than the wild strain was identified. This gene (PP_0053) encodes a putative sulfide:quinone oxidoreductase that is involved in the oxidation of glutathione demonstrating the importance of the reduced form of glutathione in the biosynthesis of nano-Se.

Avendaño R, et al. *Scientific Reports*. (2016) 6: 37155.

Martínez-García E et al. *BMC Microbiology* (2011) 11: 38.

PT005 Microcalorimetry can predict MIC-values in multidrug resistant Enterobacteriaceae

Marta Veses Garcia[1], Wilhelm Paulander[1], Magnus Jansson[1]

[1]*Symcel AB, Sweden*

Background: Monitoring the energy released during bacterial metabolism provides novel insights into their growth dynamics. Their metabolic response during antimicrobial exposure can be measured with isothermal microcalorimetry (IMC) providing a unique tool to study antibiotic phenotypic resistance.

Objectives: evaluate the ability of IMC for determining minimum inhibitory concentrations (MICs) to key antimicrobials of clinical isolates of *Enterobacteriaceae.*

Methods: 158 clinical *E. coli* and *K. pneumoniae* isolates from Sweden, Spain, Italy, and The Netherlands were tested. Of these, 37% showed resitance to piperacillin-tazobactam (PTZ), 8% to amikacin (AMK), 47% to ciprofloxacin (CIP), 14% to meropenem (MER) and 45% to cefotaxime (CTX). MICs were determined using the IMC device calScreener™ (Symcel AB, Sweden) and broth microdilution (BMD) as the reference. Essential agreement (EA), categorical agreement (CA), very major (VME), major (ME) and minor (MIE) errors for each antibiotic were determined.

Results: EA of the IMC method for PTZ, AMK, CIP, MER and CTX were 100, 98.7, 98.7, 99.3, and 98.3 respectively. CA for PTZ, AMK, CIP, MER and CTX were 99.3, 94.9, 97.5, 97.5 and 98.1 respectively. VME and ME rates of 1.3% and 1.4% were calculated for CIP and AML respectively. MiE erros range from 0.63% (PTZ) to 3.8% (AMK). To our knowledge, this is the first large scale study conducted applying IMC to clinical microbiology. IMC can accurately determine the MIC of *E. coli* and *K. pneumoniae*, including multi-drug resistant isolates, versus commonly used antimicrobials.

PT006 Microcalorimetry: a novel tool to study the metabolic effect of antimicrobial resistance

Marta Veses Garcia[1], Wilhelm Paulander[2], Magnus Jansson[2]

[1]*Symcel AB, Stockholm, Sweden*
[2]*Symcel AB, Sweden*

Background: The heat produced by metabolic reactions in a bacterial population can be measured with isothermal microcalorimetry (ICM). ICM is a sensitive technique that can detect small changes in the heat flow of a culture during the growth cycle (lag, log and stationary phase), providing a unique view into the viability and metabolic state of bacteria that traditional microbiology methods cannot give. Hence, ICM brings a new approach to the study of antimicrobial resistance by taking the metabolism of bacteria into account.

Objectives: Explore the metabolic effect of sub-inhibitory and inhibitory antimicrobial concentrations on non-fastidious bacteria.

Methods: The heat flow (metabolic response) of *E.coli, K. pneumoniae, P. aeruginosa, A. baumannii, S. aureus* and *S. epidermidis* were continuously measured using the IMC device calScreener™ (Symcel AB, Sweden) in four different media broths and exposed to the following antibiotics classes: quinolones, tetracyclines, cephalosporine, beta-lactams and carbapenems. Data collected were analysed using the calView™ software and their metabolic response were compared by calculating their growth index (GI).

Results: Analysis of the metabolic response after exposure to antibiotic on susceptible and resistant bacteria revealed: i) the metabolic effect of resistance mechanisms after antibiotic exposure. ii) The impact of antibiotic exposure in stationary phase, with the time required for mutants to arise and detection of growth of subpopulations (heteroresistance). iii) The influence of different media on metabolism, highlighting each species unique metabolic adaptation to the environment, linking it to specific carbon source use.

PT007 Design of materials to capture and enrich bacterial samples

Maria Sande[1;1], Tugce Caykara[2], Carla Silva[2], Ligia Rodrigues[1], Nuno Azoia[2]

[1]*University of Minho - Campus of Gualtar, CEB-Centre of Biological Engineering, Braga, Portugal*
[2]*CeNTI - Centre of Nanotechnology and Smart Materials, Vila Nova de Famalicão, Portugal*

Background: Diseases arising from pathogenic infections cause immense loss of life and illnesses globally besides having severe negative social and economic impact. Current methods of diagnosing such infections rely mostly on culture-based assays which are complex, time consuming and expensive. Other related problems include low detection limits of diagnostic methods, thus requiring methods to concentrate and enrich the samples being analysed.

Objectives: It is imperative that novel diagnostic methods which are simple, accurate, quick and cost-effective are developed. An approach to develop such a diagnostic platform is by including an enrichment step to concentrate the microorganisms present in the biological samples and then the detection of specific pathogens.

Methods: To capture and concentrate bacteria from samples, collagen nanoparticles were synthesised and then incubated with those samples. Collagen binds to bacteria leading to enrichment upon elution. In addition, magnetic particles were added to the collagen nanoparticles in order to facilitate the recovery of the nanoparticles from the samples.

After enrichment of a given sample a specific detection method utilizing voltammetric biosensors was used. Electrodes to be used can also be functionalised to specifically capture bacteria and avoid the adhesion of unspecific molecules to the electrode which could affect the accuracy of the read-out.

Results: Bacteria bind to the collagen-magnetic nanoparticles and these nanoparticles can be recovered using a magnetic field.

Polypropylene - which is the substrate being used for the electrodes construction – was functionalised by physical treatments including plasma, UV and ozone to ultimately produce an anti-adhesive surface.

PT008 Antibacterial activity of Uncaria tomentosa (Uña de gato) against Staphylococcus aureus and Fusobacterium nucleatum

Victor Vasquez-Tejeda[1], Ronald Aquino Ortega[2;3], Hugo Carrillo-Ng[3], Miguel Angel Aguilar-Luis[2;3], Wilmer Silva-Caso[2;3], Juana del Valle-Mendoza[2;3]

[1]*Universidad Peruana de Ciencias Aplicadas, School of Dentristry, Faculty of Health Sciences, Lima, Peru*
[2]*Universidad Peruana de Ciencias Aplicadas, School of Medicine, Research Center and Innovation of the Faculty of Health Sciences, Lima, Peru*
[3]*Instituto de Investigacion Nutricional, Molecular Biology and Biochemistry Laboratory, Lima, Peru*

Background: The use of medicinal plants is a novel alternative therapy to synthethic antibiotics because of the variety of bioactive compounds found in nature. *Uncaria tomentosa* is a native peruvian medicinal plant with antioxidant and antiinflamatory properties. However, its effect against oral pathogenic bacteria have not been studied yet. *Staphylococcus aureus* and *Fusobacterium nucleatum* are two pathogens involved in dental caries formation, with high prevalence in the oral microbiota.

Objectives: To determine the antibacterial effect of the methanol extract of *Uncaria tomentosa* (Uña de gato) against *Staphylococcus aureus* ATCC 25923 and *Fusobacterium nucleatum* ATCC 25586.

Methods: *Uncaria* tomentosa was choped and soaked in absolute methanol (1:2, w/v), then stored for 10 days. The antibacterial effect was evaluated using the cup-plate agar diffusion method, by preparing wells with the experimental solution cultivated in aerobic conditions for 24 h at 37 °C. The assay was repeated twelve times for each bacteria independently, using 0.12% chlorhexidine as a positive control. The minimum inhibitory concentration was determined using the microdilution method with brain heart infusion (BHI) broth as described by the CLSI. The cytotoxicity was determined using the MTT assay with Madin-Darby canine cells.

Results: The methanol extract showed antibacterial effect with inhibition halos of (31.3 ± 2.66) mm against *Staphylococcus aureus.* Meanwhile, clorxhexidine 0.12% had an activity of (59.1 ± 3.6) mm. The methanol extract did no show antibacterial effect against *Fusobacterium nucleatum*. The MIC against *Staphylococcus aureus was 0.0016* µg/ml. The 50% cytotoxic concentration was 366.45 µg/ml.

PT009 Exploitation of novel polymers in the design of anti-adhesive surfaces

Tugce Caykara[1;2], Maria Sande[2], Nuno Azoia[1], Ligia Rodrigues[2], Carla Silva[1]

[1]*CeNTI - Centre of Nanotechnology and Smart Materials, Vila Nova de Famalicão, Portugal*
[2]*University of Minho - Campus of Gualtar, CEB- Centre of Biological Engineering, Braga, Portugal*

Background: Bacterial infection in the healthcare sector is a rising problem. The majority of the diseases caused by bacteria are treatable using antibiotics; however, several antibiotic resistant bacteria species are emerging due to the misuse and/or overuse of antibiotics, fostering an urgent need to find alternative methods to reduce bacterial infections. Bacteria adhere to biomaterial's surfaces and further form biofilms that protect them during their colonization. Once mature bacteria leave the biofilm, they attach to a new surface and continue with the same biofilm cycle. Therefore, the bacteria adhesion exposes the patients to a higher infection risk. Hence, preventing bacterial adhesion is an interesting alternative to reduce the risk of infection. Polyetylene terephtlate (PET) and Silicone are widely used polymers by the medical industry due to their remarkable properties. However, they are hydrophobic, and it is well-known that the material hydrophilicity is an important parameter to prevent bacterial adhesion. Modification of the material's surface hydrophilicity can be obtained through its functionalization which also allows binding other relevant molecules.

Objectives: The objective of this work was to functionalize PET and silicone substrates using different methods and observe their effects on the material's properties like hydrophilicity, morphology and antiadhesive properties.

Methods: PET and silicone substrates were treated by atmospheric plasma, Ozone and UV. Modified substrates were characterized through the measurement of contact angles, their topography by SEM and anti-adhesive effect.

Results: The contact angle measurements showed that atmospheric plasma treatment with N_2 gas was effective in the functionalization of PET samples.

PT010 Propionibacteria as cell factories at near-zero growth conditions

Alexander Dank[1], Oscar van Mastrigt[1], Tjakko Abee[1], Soren Lillevang[2], Judith C. M. Wolkers-Rooijackers[1], Eddy J. Smid[1]

[1]*Wageningen University & Research, Food Microbiology, Wageningen, Netherlands*
[2]*Arla Innovation Centre, Aarhus, Denmark*

Background: In traditional food fermentation processes energy-limiting environments are often encountered by fermenting bacteria. One typical example is cheese ripening, during which for instance lactic acid bacteria remain viable for months. Microbes survive prolonged periods of energy-limitation by adapting their physiology, resulting in near-zero growth. Recently, it was shown in our laboratory that physiological adaptations to near-zero growth conditions in Lactococcus lactis result in production of characteristic secondary metabolites determining cheese aroma. Propionibacteria (PAB) are commonly used for cheese production and were found to survive harsh environments. Moreover, PAB are crucial for characteristic flavour development in Swiss-type cheeses and they display unique traits like utilization of lactate (Wood-Werkman cycle), production of propionate and production of vitamin B12, B2 and K2. The latter shows the potential of PAB as cell factories for in situ and in vitro aroma and vitamin production.

Objectives: The objective of this study is to explore the potential of PAB as cell factories. The focus will be to evaluate physiological changes under near-zero growth conditions in relation to aroma and vitamin production.

Methods: Selected PAB strains will be cultivated in a retentostat bioreactor. Subsequently, volatile aroma and metabolite production will be monitored. Finally, cell factory performance will be optimised using various approaches such a biodiversity mining, adaptive laboratory evolution and process optimisation.

Results: This study will deliver an evaluation of the potential of PAB as cell factories in bioreactor cultivations for aroma and vitamin production.

PT011 Graphene oxide regulates secondary metabolism in pseudomonads and streptomycetes

Adrian Augustyniak[1;2], Krzysztof Cendrowski[3], Martyna Trukawka[3], Paweł Nawrotek[1]

[1]*West Pomeranian University of Technology, Szczecin, Department of Immunology, Microbiology, and Physiological Chemistry, Szczecin, Poland*
[2]*Technische Universitaet Berlin, Department of Civil Engineering, Berlin, Germany*
[3]*West Pomeranian University of Technology, Szczecin, Nanomaterials Physicochemistry Department, Szczecin, Poland*

Background: Nanomaterials are used in microbiology mostly as antimicrobials. Although their toxicity seems to be well studied, there is sparse information on the effects that these substances have on bacterial secondary metabolism. Recent findings show that the oxidative stress caused by nanomaterials can be transformed in bacterial cells to increase the production of adenosine triphosphate. Investigating the interaction between bacteria and novel nanomaterials in terms of the activation (or inhibition) of secondary metabolism can provide novel solutions for the production of significant metabolites.

Objectives: The aim of this study was the evaluation of effects caused by graphene oxide on the activity of secondary metabolism in bacteria from genera *Pseudomonas* and *Streptomyces*.

Methods: Two strains of *P. aeruginosa* and five strains of *Streptomyces* spp. were contacted with graphene oxide and graphene oxide functionalised with cobalt nanoparticles. Physiological parameters, metabolic activity (respiration), secretion of pigments, and antagonistic behaviour were examined. Study was performed with spectrophotometry, flow cytometry, liquid chromatography and mass spectrometry.

Results: Graphene oxide caused shifts in the secondary metabolism of tested bacteria. In pseudomonads, the production of pyocyanin was highly upregulated, whereas streptomycetes changed the pattern of antagonistic behaviour against reference microorganisms, depending on the type of used nanomaterial. Furthermore, graphene oxide functionalised with cobalt nanoparticles induced one streptomycete to produce an antifungal agent in the liquid culture. Gathered evidence shows that nanomaterials could be potentially used as stimulants in the production of secondary metabolites.

PT012 Production of a Functional Recombinant Reductive Dehalogenase

Rabeya Rahmatullah[1], Bat-Erdene Jugder[2], Matthew Lee[3], Haluk Ertan[3], Christopher Marquis[1]

[1]*UNSW Sydney, School of Biotechnology and Biomolecular Science, Sydney, Australia*
[2]*Harvard Medical School, Boston, United States*
[3]*UNSW Sydney, School of Civil and Environmental Engineering, Sydney, Australia*

Background: Reductive dehalogenase enzymes (RDases) in Organohalide Respiring Bacteria (OHRB) are key enzymes responsible for detoxification of organohalides, which are recalcitrant environmental pollutants. Because of their potential role in organohalide bioremediation, improving the understanding of their structure-function relationship is of great interest. Heterologous expression provides a means by which larger amounts of these enzymes can be generated for such studies. The *Dehalobacter* strain UNSWDHB is capable of respiring chloroform (CF) mediated by the reductive dehalogenase, TmrA, which has been purified in its native form and expressed in *Bacillus megaterium* with limited catalytic activity.

Objectives: This study aims at evaluating recombinant production of TmrA in *Shimwellia blattae* with specific activity comparable to native enzyme and an increase in soluble expression of TmrA by co-expressing it with a chaperone protein.

Methods: Tagged and codon optimized *tmrA*, devoid of its TAT-signal, was cloned into pASK-IBA63c-plus vector and transferred into competent *S. blattae* cells. After anhydrotetracycline-inducible expression of TmrA, the cells were chemically lysed under anaerobic conditions and the CF dechlorination activity of soluble protein fraction was measured by gas chromatography/mass spectrometry using Titanium (III) citrate and methyl viologen as electron donors. Similar experiments were performed with full TmrA and co-expression of host chaperones.

Results: TmrA has been expressed in soluble form in *S. blattae* and CF dechlorination activity has been observed *in vitro*. The heterologous expression of TmrA in *B. megaterium* and *S. blattae* confirms the requirement of a host capable of cobalamin synthesis and/or uptake for production of active RDases.

PT013 Antibacterial efficiency of surface immobilized Flavobacterium infecting bacteriophage

Miika Leppänen[1], Ilari Maasilta[2], Lotta-Riina Sundberg[3]

[1]*University of Jyvaskyla, Department of physics, Department of biological and environmental science*
[2]*University of Jyvaskyla, Department of physics*
[3]*University of Jyvaskyla, Department of biological and environmental science*

Background: Bacterial disease control by bacteriophages (phages) has gained recently more interest due to antibiotic resistance. Immobilisation of phages on materials can be used to provide a local antibacterial effect.

Objectives: Immobilisation approach has been already successfully applied in a few studies, but the basis of the antibacterial effect has remained unclear and coherence in the interpretation of results is poor.

Methods: We studied phage attachment density, detachment rate and infectivity on different surfaces: silicon, amine treated silicon, gold, carboxylate treated gold and crosslinker-activated carboxylate treated gold. To understand whether antibacterial effect is caused by the attached or detached phages, the strength of the immobilization was analysed by 3-12 washing steps.

Results: The density of attached phages varied between surfaces, being highest on the crosslinker-activated carboxylate-treated gold. Detachment differed between the materials and amine surface with the high release of phages maintained highest infectivity even after extensive washing. Covalent crosslinking of phage seemed to interfere the infectivity. Our results suggest that the detachment of the phages from the surface should be considered as a possible mechanism for the antibacterial effect by the immobilised phages. Furthermore, we suggest measuring infectivity of the immobilised phages by comparing the bacterial growth reductions by the material and by known number of free phages, resulting to “Effective PFU/surface area” as a comparable unit between studies.

PT014 Can plant-associated bacteria from an uranium mine have biotechnological interest for nanopolymer biosynthesis?

Catarina Marques[1], Der-Shyan Sheu[2], Amadeu M.V.M. Soares[1]

[1]Universidade de Aveiro, Departamento de Biologia & CESAM (Centre of Environmental and Marine Studies), Aveiro, Portugal
[2]National Kaohsiung Marine University, Department of Marine Biotechnology, Kaohsiung, Taiwan

Background: The resilience of bacteria inhabiting uranium mine areas is supported by mechanisms they evolved to withstand harsh conditions regarding chemical and radiological contamination, as well as limiting nutrient loads. One of the metabolic strategies developed relies on the creation of energy storages by the production and accumulation of nanopolymers, like polyhydroxyalkanoates (PHA), which value has been suggested towards different biotechnological purposes.

Objectives: To disclose the ability of rhizoplane and endophytic bacteria isolated from the roots of plants (*e.g.*, *Pinus pinaster*, *Salix atrocinera*) collected in an abandoned uranium mine (Cunha Baixa,Portugal) for the biosynthesis of PHA nanoparticles with potential biotechnological value.

Methods: PHA-producing bacteria were screened through two sequential methods: (i)use of culture media with C enriched sources and vestigial N/P, followed by microscopic monitoring of PHA synthesis; (ii)molecular (PCR-based) confirmation with sets of primers targeting conserved regions of PHA synthases, which amplicons were sequenced. The nanopolymers synthesized by the most-producing bacteria were isolated and microscopically characterized.

Results: No great variability was recorded on the number of PHA-producing bacteria between plant species. Hence, from a total of 99 endophytes and 116 rhizoplane bacteria, 19% and 23% were respectively selected through culture-dependent/microscopic methods, as PHA-producing bacteria. However, the presence of PHA synthases-encoding genes was molecularly confirmed for a low amount of bacteria. The microscopic characterization of PHA nanoparticles produced per cell evidenced similar number, size and shape. Their biocompatible and biodegradable features are attractives for environmental, industrial and medical biotechnological applications.

PT015 Detection of novel antimicrobial resistance genes in oil contaminated soils by metagenomic functional analysis

Amando Flores[1], Eva María Camacho[1], Eduardo Santero[1]

[1]*Centro Andaluz de Biología del Desarrollo/Universidad Pablo de Olavide/CSIC/Junta de Andalucía, Seville, Spain*

Background: Our ability to cure infections that were once considered benign or of low risk is at stake due to the development and spread of antimicrobial resistance mechanisms (AMR). One of the measures proposed to fight this problem is the research aimed at detection and molecular characterisation of the AMR to design strategies to combat them. In this regard, metagenomic functional analyses are powerful tools to detect new AMR genes in different environments.

Objectives: In this work, we have searched for genes that confer resistance to different antibiotics used in hospitals by following a metagenomics approach.

Methods: We have screened two metagenomic libraries, previously constructed in our laboratory, from two different locations. In order to overcome the inherent limitation of expressing heterologous genes from long DNA fragments in *E. coli*, we have used a fosmid vector that allows processive transcription of long stretches of environmental DNA by using two viral transcriptional machineries: (1) the phage T7 RNA-polymerase, which is insensitive to many of the bacterial transcription terminators and (2) the use of the lambda N-anti-termination protein coupled to a salicylate inducible promoter. We have searched for fosmids from both libraries that are resistant to different groups of antibiotics used in hospitals.

Results: Although we could not find any fosmid conferring resistance to polimixins or fluoroquinolons, we have detected clones resistant to cephalosporins and carbapenems bearing fosmids that code for extended spectrum □-lactamases, metallo-□-lactamases and/or efflux pumps.

Supported by Spanish Ministry of Science, Innovation and Universities grant SAF2017-85785-R

PT016 Serine phosphorylation in FTSZ protein as a key to understand Streptomyces development

Paula Yagüe[1], joost willemse[2], angel manteca[3], Gilles van Wezel[4]

[1]*institute of Biology, microbiotechnology and health, Netherlands*
[2]*institute of biology, microbiotechnology and health, Netherlands*
[3]*microbiology area, fucntional biology, Spain*
[4]*institute of biology, microbiotechnology and health, Netherlands*

Background: *Streptomyces* is a bacterial genus with a complex life cycle, that produce a plethora of bioactive compounds.,.. During sporulation-specific cell division in streptomycetes, up to a hundred rings of the tubulin like FtsZ are produced simultaneously, dividing the multinucleoid aerial hyphae into chains of unigenomic spores. Uniquely, *ftsZ* is not essential for growth of *Streptomyces*. A recent discovery in our laboratory is that FtsZ is also produced at high levels during the earliest stages of *Streptomyces* growth. At this stage, it is involved in the formation of membranous septa (without peptidoglycan). FtsZ was shown to be phosphorylated in two serine sites and these post-translational modifications are much more prevalent during sporulation than in the early stages of growth.

Objectives: To obtain insights into the role of the post-translational serine phosphorylation of FtsZ during the *Streptomyces* life cycle

Methods: The methodology used for this study has been the construction of four *Streptomyces* mutants, wherein the serine residues have been changed to residues that are either never pohosphorylated (alanine) or that are always phosphorylated (glutamic acid). In this way, we have been able to obtain derivatives with different degrees of phosphorylation, and study the impact of these changes on the function of FtsZ. This allowed us to study the consequences of FtsZ phosphorylation on the development of *Streptomyces*.

Results: The analysis by different methodologies of these four mutants has resulted in highly different phenotypes regarding growth, morphogenesis, sporulation and antibiotic production. The most recent data will be discussed.

PT017 Cave bacteria in the fight against lung cancer and associated microbial infections

Diana Pereira[1], Amadeu M.V.M. Soares[1], Catarina Marques[1]

[1]*Universidade de Aveiro, Departamento de Biologia & CESAM (Centro de Estudos do Ambiente e do Mar), Portugal*

Background: Lung cancer leads the cancer-related mortality rate worldwide. Conventional therapeutics have limitations in terms of efficacy, and drug resistance, which are often worsened by the occurrence of bacterial respiratory tract infections (BRTI). Hence, the search for new bacterially-derived natural compounds with anticancer and/or antimicrobial activity is crucial, being under-explored environments a source of biosynthetic bacteria with unrated biotechnological potential.

Objectives: (1)To screen the antimicrobial activity of bacteria isolated from caves; (2) to evaluate the antimicrobial and anticancer activity of cave bacteria crude extracts.

Methods: Cave bacteria were isolated from different environmental niches in limestone caves. Their antimicrobial activity was primarily screened against BRTI (*Escherichia coli*, *Micrococcus luteus*, *Aeromonas hydrophila*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*) by the spot-on-lawn method. Bioactive cave isolates were identified by 16S rRNA gene sequencing, and their crude extracts were then prepared through the use of ethyl acetate and/or acetone. Crude extracts (cell-free and cell fractions) antimicrobial activity was confirmed by the disc-diffusion assay against BRTI, whilst their cytotoxicity was evaluated against murine Lewis lung cancer (LLC) cells by the colorimetric MTT assay.

Results: Among 264 cave bacteria, 14% presented antimicrobial activity against BRTI, being mainly identified as *Pseudomonas* species (68%). However, the corresponding crude extracts showed a slightly decreased antimicrobial activity, in comparison to that recorded in the live-colony spot-on-lawn assay. Plus, the anti-BRTI activity of cell and cell-free fractions was different. The MTT assay, though, demonstrated that 73% of the crude extracts (cell and cell-free fractions) had significantly inhibited the activity of LLC cells.

PT018 Comprehensive characterization of l-asparaginase from lactic acid bacteria

Kodchakorn Phetsri[1], wakayama mamoru[1]

[1]*Ritsumeikan university BKC, Biotechnology, Shiga, Japan*

Background: L-asparaginase (ASNase) which catalyzes L-asparagine (L-Asn) to be L-aspartic acid (L-Asp) and ammonia is the essential enzyme in L-asparagine metabolism. ASNase has been effectively used for acute lymphoblastic leukemia (ALL) treatments and the reducing acrylamide formation in processing starchy food. Lactic acid bacteria (LAB) play important roles for human health and the food industry. However, the commercial LASNases from *Escherichia coli* and *Erwinia chrysanthemi* have been mainly applied to medical field. Only ASNases from *Aspergillus* sp. are currently used for food application.

Objectives: The aims of this study was to clone ASNase gene from LAB and to investigate the properties of the recombinant ASNase.

Methods: LABs exhibiting ASNase activity were screened by GlDH assay method to detect ammonia released from L-Asn. Among LABs examined, *L. acidophilus* NBRC 13951 was selected, cloned ASNase gene and expressed in *E. coli*. LaASNase was purified using the ion exchange chromatography. The properties such as pH optima, temperature stability, and kinetic analysis of LaASNase have been examined.

Results: LaASNase gene (1kbps) was obtained by PCR and inserted into *XhoI* and *NdeI* sites of pET-28a. *E. coli* BL21 (DE3) harboring. LaASNase was purified with the specific activity of 17.60 U/mg and 13.30 folds. The purity of the enzyme was confirmed by SDS–PAGE with a molecular weight of 37.5 kDa.

Conclusions: The study reveals the desirable property of LaASNase which is possibly applied to be a food production. However, the preservation time of the enzyme is slightly short and would be studied afterward.

PT019 Scarless gene knockout system for iterative engineering of Corynebacterium glutamicum

Kyeong Rok Choi[1], Jae Sung Cho[1], Cindy Pricilia Surya Prabowo[1], Jae Ho Shin[1], Dongsoo Yang[1], Jaedong Jang[1], Sang Yup Lee[1]

[1]*Korea Advanced Institute of Science and Technology, Department of Chemical and Biomolecular Engineering, Daejeon, Korea, Rep. of South*

Background: *Corynebacterium glutamicum* is an important industrial microorganism for production of amino acids. Genome engineering of *C. glutamicum*, however, currently relies on random mutagenesis and inefficient homologous recombination through double crossover events. Development of efficient genome engineering tool for *C. glutmicum* is required.

Objectives: We report a rapid and scarless genome engineering strategy for sequential and iterative knockout of one or more genes in *C. glutamicum*.

Methods: Recombinase RecT derived from *E. coli* Rac prophage was used to incorporate single-stranded oligodeoxyribonucleotides to the chromosome of *C. glutacmicum*. In addition, CRISPR/Cas9 system was combined to counter-select cells of which target chromosomal locus is unmodified. The scarless and markerless system was completed by developing plasmid curing strategies for the RecT and CRISPR/Cas9 vectors.

Results: The scarless genome engineering system was demonstrated by constructing seven different recombinant *C. glutamicum* strains with combinatorial deletion of three different target genes related to the production of γ-aminobutyric acid, an industrially important chemical. In addition, konckout of two non-contiguous genes could be successfully deleted simultaneously while selecting the modification of only one of the two target genes. Our genome engineering strategy will expedite metabolic engineering of *C. glutamicum* for production of industrially important chemicals [This work was supported by the Technology Development Program to Solve Climate Changes on Systems Metabolic Engineering for Biorefineries (NRF-2012M1A2A2026556 and NRF-2012M1A2A2026557) from the Ministry of Science and ICT, through the National Research Foundation (NRF) of Korea. This work was further supported by Hanwha Chemical through KAIST-Hanwha Chemical Future Technology Institute.]

PT020 Secretory production of free heme from metabolically engineered Escherichia coli

Kyeong Rok Choi[1], Xin Rui Zhao[1], Sang Yup Lee[1]

[1]Korea Advanced Institute of Science and Technology, Department of Chemical and Biomolecular Engineering, Daejeon, Korea, Rep. of South

Background: Heme has various applications in healthcare and food supplement industries. Current production of heme, however, relies on complex, low-yielding, and eco-unfriendly organic extraction from animal bloods/plants and chemical synthesis. *Escherichia coli* has previously been engineered to produce heme through the C4 pathway and provide an alternative route for heme production, but only a small amount was accumulated inside the cell, requiring subsequent extraction step for further uses.

Objectives: We engineered *E. coli* to produce and secrete significant amount of free heme to the medium, which simplifies recovery of the product for subsequent applications.

Methods: Endogenous C5 pathway for 5-aminolevulinic acid (ALA) biosynthesis was enhanced and downstream heme biosynthetic pathway was optimized to increase the production of heme. In addition, *ldhA*, *pta*, and also *yfeX* (encouding a putative heme-degrading enzyme) genes were knocked out for further optimization. Moreover, endogenous heme exporter genes *ccmABC* were overexpressed to facilitate export of free heme produced.

Results: The engineered *E. coli* strain secretes 73.4 and 151.4 mg l^{-1} heme, which are 63.5 and 63.3% of 115.5 and 239.2 mg l^{-1} total heme produced from glucose only and glucose supplemented with L-glutamate, respectively, in fed-batch fermentations. Our engineered *E. coli* strain and the engineering strategies will be useful for microbial secretory production of free haem. [This work was supported by the Technology Development Program to Solve Climate Changes on Systems Metabolic Engineering for Biorefineries (NRF-2012M1A2A2026556 and NRF-2012M1A2A2026557) from the Ministry of Science and ICT, through the National Research Foundation (NRF) of Korea]

PT021 Microbial production of enzymes from blood and pulp processing waste streams

Renate Weiss[1], Gibson Stephen Nyanhongo[1], Georg Gübitz[1]

[1]*Universität für Bodenkultur Wien-Department IFA-Tulln-Int. Univers. Forschungsinstitut, Agrobiotechnology, Tulln an der Donau, Austria*

Background: The pulp and paper industry as well as the meat processing industry incur huge costs for the treatment and safe disposal of their produced waste streams. Therefore, creating technologies for waste utilization to produce high value products does not only save costs but also creates profits for the respective industries

Objectives: The aim of this study is to develop cost effective enzyme production systems for in-house application utilizing deinking sludge from the Pulp and Paper Industry as well as nutrient rich blood waste from the meat processing industry. *Trichoderma reesei* (cellulase), *Candida cylindracea* (lipases) and *Bacillus licheniformis* (proteases) were chosen as natural enzyme producers.

Methods: To produce the desired biocatalysts, the carbon and nitrogen source of the production media was replaced by cellulose fibers from deinking sludges and porcine blood. The culture supernatants were analyzed via the respective enzyme assays.

Results: The microbes generally grew well when pasteurized blood and deinking sludges were added to the standard media.

T. reesei showed superior enzyme production when the nitrogen source was substituted, the substitution of the carbon source didn`t lead to the desired results.
Substitution of the nitrogen source in the other Organisms was tested with blood plasma, pasteurized blood and sterilized blood. *C. cylindracea* was not able to produce Lipases when the nitrogen source was substituted. *B. licheniformis* produced alkaline proteases, when substituted with blood plasma and sterilized blood.

PT022 Optimization of alkaline sugarcane bagasse pre-treatment for the production of 2nd generation bioethanol

Adebare Adeleke[1;2], Hayatu Raji[3], Dimitris Hatzinikolaou[4], Sunday Odunfa[1]

[1]*University Of Ibadan, Department of Microbiology, Ibadan, Nigeria*
[2]*Modibbo Adama University of Technology, Yola, Department of Microbiology, Yola, Nigeria*
[3]*American University of Nigeria, Department of Natural and Environmental Science, Yola, Nigeria*
[4]*National and Kapodistrian University of Athens, Enzyme and Microbial Biotechnnology Unit, Department of Biology, Athens, Greece*

Background: Bioethanol from lignocellulosic wastes represents a promising alternative to fossil fuels. The process involves enzymatic hydrolysis of cellulose and hemicellulose, followed by the fermentation of the resulting hexoses and pentoses to ethanol. The recalcitrant nature of lignocellulose though, requires the employment of a pre-treatment step that loosens the complex cellulose-hemicellulose matrix and facilitates enzymatic attack. Alkaline pre-treatment is a commonly used pre-treatment, since it effectively removes lignin with little effect on the carbohydrates.

Objectives: In the present work, we optimized sugarcane bagasse (SB) alkaline pre-treatment using potassium hydroxide (KOH) and evaluated the capacity of *Candida tropicalis* Y5 (Accession number MG321586) to produce ethanol from pre-treated SB enzymatic hydrolysate.

Methods: SB alkaline pre-treatment was optimized with respect to KOH load, treatment time, and temperature, using response surface methodology (RSM). The objective function was the sugar yield after each pre-treatment condition, following enzymatic hydrolysis with a commercial cellulase cocktail. Fermentation of the optimally pre-treated material was carried out through separate hydrolysis and fermentation (SHF) with *C. tropicalis* Y5.

Results: Optimum pretreatment conditions were found to be KOH load at 150mg/g bagasse, 86^{o}C and 120 minutes of pre-treatment time. Maximum total reducing sugar of 600 mg/g bagasse was observed. Ethanol yield was 19g/L with fermentation efficiency of 98% (calculated on hexose content). Our results show that pre-treatment of SB using low concentrations of KOH enhanced enzymatic hydrolysis without production of inhibitors (acetic acid and furfural). *Candida tropicalis*Y5 showed moderate tolerance to stress factors and successfully converted hexose and pentose sugars to ethanol.

PT023 Separation and purification of three, four, and five carbon diamines from fermentation broth

Jong An Lee[1], Jung Ho Ahn[1], Inho Kim[1], Sheng Li[1], Sang Yup Lee[1]

[1]KAIST, Department of Chemical and Biomolecular Engineering, Daejeon, Korea, Rep. of South

Background: 1,3-diaminopropane (1,3-DAP), 1,4-diaminobutane (1,4-DAB), and 1,5-diaminopentane (1,5-DAP) are important chemicals due to their wide industrial use including agrochemicals, pharmaceuticals, surfactants, and building blocks for nylon synthesis. Also, several studies on bio-based production of diamines by metabolically engineered microorganisms have been published. However, development of efficient methods for the recovery of 1,3-DAP, 1,4-DAB, and 1,5-DAP from fermentation broth has not been reported.

Objectives: Development of effecient separation and purification process of 1,3-DAP, 1,4-DAB, and 1,5-DAP from fermentation broths.

Methods: The optimal process for the recovery and purification of 1,3-DAP, 1,4-DAB, and 1,5-DAP from fermentation broth comprises several unit operations including removal of cell debris, decolorization of fermentation broth, product concentration, deprotonation of diamines, product separation, and final polishing.

Results: This study is the first to report successive separation and purification of pure 1,3-DAP from a fermentation broth. The technical feasibility of the newly designed simple diamines separation and purification from fermentation broths was tested and pure 1,3-DAP, 1,4-DAB, and 1,5-DAP were obtained with recovery yields of 87 ± 3%, 86 ± 4%, and 81 ± 2%, respectively. The strategy employed in this study will be applicable in developing downstream processes to recover other diamines from fermentation broths. Furthermore, this work can be used as a basis for the development of bio-based nylon industry. This work was supported by the Technology Development Program to Solve Climate Changes on Systems Metabolic Engineering for Biorefineries (NRF-2012M1A2A2026556 and NRF-2012M1A2A2026557) from the Ministry of Science and ICT through the National Research Foundation (NRF) of Korea.

PT024 Development of genetic tools for bacterial engineering

Sara Baldanta Callejo[1], María Castillo López[1], Flor Govinda Guevara[1], Patricia Suárez Rodríguez[2], María Navarro Llorens[1]

[1]UCM (Complutense University of Madrid), , Department of Biochemistry and Molecular Biology I, Madrid, Spain
[2]UCM (Complutense University of Madrid), Department of Biochemistry and Molecular Biology I, Madrid, Spain

Background: Currently, there is a wide toolbox for the modification of 'model' bacteria. However, other species of interest lack of proper tools, a step essential for displaying their full biotechnological potential.

Two of these species are *Rhodococcus* and cyanobacteria that still have some constrictions in their genetic engineering, such as the few characterized *Rhodococcus* promoters and the evasion of 'host barriers' in cyanobacteria to allow the transformation process.

Objectives: The aims of this work are: i) the study of promoters and expression of the different isoforms of an enzyme involved in the steroids degradation pathway, the 3-ketosteroid-9α-hydroxylase (Ksh) in *Rhodococcus ruber* strain chol-4; ii) the identification and characterization of the foreign DNA barrier systems present in some cyanobacteria in order to prepare the DNA to be used in the transformation process.

Methods: The *ksh* putative promoters were cloned and characterized together with *Rhodococcus* growth studies, accumulation of metabolic intermediates and determination of *Ksh*'s expression on different substrates.

An extensive search of the restriction barriers and CRISPR elements were done in *Arthrospira* and the heterologous expression of all the methyltransferases found to allow *in vitro* methylation were performed.

Results: The putative *kshs* promoters in strain Chol-4 probed to be functional displaying differences depending on the substrate. These promoters could be useful for further promoter engineering broadening the genetic tools for *Rhodococcus*.

Arthrospira contains several restriction barriers, including 13 methyltransferases of type II. Six of them exhibited activity in *E. coli* and other 5 were active in *Rhodococcus erytropolis*.

PT025 Paraburkholderia xenovorans LB400 flavodoxins are relevant in the resistance to the redox-cycling compound paraquat

Laura Rodriguez Castro[1], Roberto Durán[1], Valentina Méndez[1], Michael Seeger[1]

[1]*Universidad Técnica Federico Santa María, Laboratorio de Microbiología Molecular y Biotecnología Ambiental, Departamento de Química, Valparaiso,*

Background: *Paraburkholderia xenovorans* LB400 is a model bacterium to study the degradation of polychlorinated biphenyls and aromatic compounds. The aerobic metabolism of aromatic compounds generates reactive oxygen species and oxidative stress. Flavodoxins are small electronic transfer proteins that participate during oxidative stress in cell redox balance.

Objectives: The aim is to characterize the protective effects of *P. xenovorans* LB400 flavodoxins during oxidative stress.

Methods: Bioinformatic tools were used to identify genes encoding flavodoxins in the genome of *P. xenovorans* LB400. Two genes encoding flavodoxins were identified (FldA and FldB) and overexpressed in *P. xenovorans* LB400. The sensitivity to 20 mM paraquat was studied. Survival assays in presence of 1 and 20 mM paraquat were performed with the recombinant *P. xenovorans* strains. Lipid peroxidation were determined measuring the thiobarbituric acid reactive substances (TBARS) after incubation with 1 mM paraquat. Protein carbonylation was determined after exposure to 1 mM paraquat.

Results: Strain LB400_FldA showed higher resistance than the control strain to 20 mM paraquat in plate assays. Both recombinant strains exhibited higher survival after exposure to 1 mM paraquat compared to control strain, whereas strain LB400_FldA displayed a higher survival with 20 mM paraquat. Strain LB400_FldB displayed lower TBARS content after incubation with 1 mM paraquat than control strain. No significant differences in protein carbonylation between recombinant and control cells after exposure to 1 mM paraquat were observed. In conclusion, the flavodoxins of *Paraburkholderia xenovorans* LB400 confers protection to oxidative stress produced by the redox-cycling compound paraquat.

PT026 How the design of filter media affects hydrodynamics and biofilm morphology in drinking water

Erifyli Tsagkari[1], Stephanie Connelly[1], Zhaowei Liu[1], Andrew McBride[1], William Sloan[1]

[1]*University of Glasgow, School of Engineering, Glasgow, United Kingdom*

Background: Biofilms play an essential role in treating water in bio-filtration systems. The biofilm morphology and function are inextricably linked to the hydrodynamics of flow through a filter and yet engineers rarely explicitly engineer this interaction. Here, we develop a system that links 3-D printing and computer simulation to optimize filter media shape and biofilm function.

Objectives: We design and optimise filter media to produce von Karman vortices that roll down a surface, imposing a shear stress that shapes biofilm morphology. Experimental and modelling results are combined to assess the effect of these vortices created due to the filter media on biofilms.

Methods: A flow channel was 3-D modelled and an object with varying geometry, which represented the filter medium, was constructed within it in order to create disturbances in the flow. After the flow channel was 3-D printed, a slide was placed attached to this object and represented the available surface for biofilm formation. A recirculation mode of flow of drinking water was operating for 24 hours. Using fluorescence microscopy, information about the morphology and growth of biofilms was acquired. Spatial statistics were used to characterize the biofilm structures. Finally, visualization of the patterns of vortices was achieved experimentally and using a 2-D computational model.

Results: The statistical analysis indicated that more extended and heterogeneous biofilms were formed for the stronger von Karman vortices. Overall, it was found that von Karman vortices created by the filter media play a key role in shaping biofilms in drinking water.

PT027 Direct measure of the effect of Isoniazid on M. smegmatis using acoustic trapping combined with wavelength modulated Raman spectroscopy

Vincent Baron[1], Mingzhou Chen[2], Björn Hammarström[3], Peter Glynne-Jones[4], Stephen Henry Gillespie[1], Kishan Dholakia[2]

[1]University of St Andrews, School of Medicine, St Andrews, United Kingdom
[2]University of St Andrews, School of Physics and Astronomy, St Andrews, United Kingdom
[3]KTH Royal Institute of Technology, Applied Physics, Stockholm, Sweden
[4]University of Southampton, Engineering, Southampton, United Kingdom

Background: Tuberculosis (TB) remains a leading cause of death worldwide due to an infectious disease. Relapse represents a major obstacle to reduce the length of TB treatment. Mycobacteria develop lipid inclusions in response to various stresses. Lipid rich, phenotypically antibiotic tolerant, bacteria are more resistant to antibiotics and may be responsible for relapse and the need for long-term treatment.

Objectives: The aim of this work is to study antibiotic effects and lipid content of mycobacteria over time.

Methods: We have developed a microfluidic system that allows us to trap live *M. smegmatis* in a chamber with acoustic waves and acquire measurements over time using wavelength modulated Raman spectroscopy (WMRS). In this system multiple parameters (chamber temperature, laser power, amplitude and frequency of the acoustic trapping…) can be controlled and recorded. Trapped mycobacteria were interrogated, continuously, in fresh medium and also in presence of the antibiotic, isoniazid, for up to 8 hours.

Results: The Raman profile of *M. smegmatis* changed substantially compared to the control following exposure to isoniazid; lipid peaks increased over time starting within the first hours of contact. This result suggests that isoniazid causes stress that leads to an accumulation of lipids. Thus, our innovation allows us to monitor bacteria in real time taking, for example, Raman measurements over more than 20 hours. This tool can be used to study a large variety of microorganisms and monitor the response to different conditions and stimuli such as the effect of different media, molecules, and other antibiotics.

PT028 Characterization of the biotransformation of hexachlorocyclohexanes during uptake into plant - first step towards for a food web study

Liu Xiao[1], Wu Langping[1], Richnow Hans Hermann[1]

[1]*Helmholtz-Zentrum für Umweltforschung – UFZ, Department of Isotope Biogeochemistry, Leipzig, Germany*

Background: Hexachlorocyclohexane isomers (HCHs) are persistent organic chemicals. The global use of Lindane (γ-HCH) and the widespread of HCH residuals caused contamination in soil and uptake into plants. The uptake of HCHs from soil to plant is the first step to enter the food web. For sustainable management of contaminated sites and risk assessment, the understanding of the transformation of HCHs in soil and plant system in the food web is essential.

Objectives: To characterize the biotransformation of HCHs in bulk soil, rhizosphere and plant

To identify the soil microbial community pattern in rhizosphere

Methods: Compound specific isotope fractionation

Enantiomer fractionation

Metagenomics

Results: We studied the biotransformation of HCHs in soil and plant to characterize natural reactive transport process using pot experiment with HCH-contaminated soil and wheat as a model system.

Concentration of soil and plant. The concentration of α-HCH in bulk soil was significantly higher than in soil rhizosphere, but there is no significant difference for β-HCH. α-HCH and β-HCH accumulated in different wheat tissues.

Isotope fractionation. The carbon isotope fractionation ($\delta^{13}C$) and chlorine isotope fractionation ($\delta^{37}Cl$) of α-HCH increased in soil rhizosphere compared to bulk soil suggesting microbial biotransformation. The $\delta^{13}C$ and $\delta^{37}Cl$ of α-HCH increased in plant tissues compared to soil samples suggesting further transformation in wheat. In contrast, there was no difference of isotope composition of β-HCH in plant and soil samples indicating β-HCH was not be transformed.

Enantiomer fractionation. The preferential transformation of specific enantiomer of α-HCH in soil and plant suggested a biological mechanism.

PT029 Optimizing locked nucleic acid/2'-o-methyl-rna fluorescence in situ hybridization (lna/20ome-fish) procedures for bacterial detection

Andreia Azevedo[1;2;3;4], Ricardo Fernandes[5], Nuno Azevedo[5], Carina Almeida[6]

[1]*Centre of Biological Engineering, University of Minho, Braga, Portugal*
[2]*FEUP - Faculdade de Engenharia da Universidade do Porto, Laboratory for Process Engineering, Environment, Biotechnology and Energy, Porto, Portugal*
[3]*IPATIMUP - Instituto de Patologia e Imunologia Molecular da Universidade do Porto, Porto, Portugal*
[4]*i3S - Instituto de Investigação e Inovação da Universidade do Porto, Porto, Portugal*
[5]*FEUP - Faculdade de Engenharia da Universidade do Porto, Porto, Portugal*
[6]*INIAV - Polo de Vairão, Vairão, Portugal*

Background: Despite the successful application of locked nucleic acid/2'-O-methyl-RNA fluorescence *in situ* hybridization (LNA/2'OMe-FISH) procedures for bacteria detection, there is a lack of knowledge on the properties that affect hybridization. Such information is crucial to find the more suitable hybridization conditions for bacteria detection either in an individual or in a multiplex assay.

Objectives: This work aimed to evaluate the effect of three essential factors on the LNA/2'OMe hybridization step - hybridization temperature, [NaCl] and type/concentration of denaturant (formamide, ethylene carbonate and urea).

Methods: The optimization was performed for 3 Gram-negative bacteria (*Escherichia coli* CECT 515, *Citrobacter freundii* SGSC 5345 and *Pseudomonas aeruginosa* PAO1) and 2 Gram-positive bacteria (*Enterococcus faecalis* CECT 184 and *Staphylococcus epidermidis* RP61A), using an Eubacteria LNA/2'OMe probe (5'mTlGlCmClTmCmClCmGmTlAmGmGlA3'; "l" - LNA; "m" – 2'OMe). The signal quantification was evaluated by flow cytometry and Response Surface Methodology was used to model the interaction between the 3 parameters.

Results: It was observed that a high NaCl concentration is beneficial (2M-5M), regardless of the denaturant used. Urea, formamide and ethylene carbonate are suitable denaturants for LNA/2'OMe-FISH applications; but urea provides higher fluorescence intensities among the different bacteria. The results indicate that a hybridization solution with 2M of urea and 4M of NaCl would be a proper starting point for multiplex LNA/2'OMe-FISH procedures. Furthermore, a hybridization temperature around 62°C, for 14bp probes with LNA monomers at every third position of 2'OMe might be use in initial optimizations.

PT030 Stabilization of microbial samples for unbiased DNA and RNA retrival

Stefan Thiele[1], Adrian Schleeh[1], Jens Van Der Flierdt[2], Dagmar Herold[1], Dominic O'Neil[1]

[1]*Qiagen GmbH, Dept. of Microbiome Product Development, Hilden, Germany*
[2]*Qiagen GmbH, Dept. of CTC Product Development, Hilden, Germany*

Background: Storing and transporting samples without changes in microbial community representation is a common challenge in sampling campaigns. This challenge affects studies in a wide variety of settings and sample types, e.g Stool samples are exposed to oxygen, water samples are subject to bottle effects, and physicochemical parameter change for soil samples. These changes effect the microbial community and consequently the results of microbial analysis. Therefore, optimal sample storage will preserve the microbial community at the time of sampling, and maintain that community over a period of time sufficient to enable analysis.

Objectives: Investigate the stabilizing effects of sample preservation solutions and other methods for whole cells or cell lysates preservation to develop a method for optimal long term sample storage.

Methods: We tested different commercially available sample storage solutions for whole cells and their effect on the DNA and RNA quality during a time series of 7 days at 37°C to mimic long term sample storage. In addition, we tested several cell lysing solutions and their effect on DNA and RNA in a similar set up. Subsequently, the DNA and RNA was extracted and analysed using gel electrophoresis, fluorimetric quantification, and 16S rRNA sequencing.

Results: We present here the results of different stabilization methods on microbial DNA and RNA from fresh stool and soil samples in comparison to sample storage in sterilized water as negative control. Best practices for sample stabilization and storage with these methods were determined, and will be presented.

PT031 Development of a microbial factory for the production of aromatic polyesters from glucose

So Young Choi[1], Jung Eun Yang[2], Si Jae Park[3], Won Jun Kim[1], Hyeong Jun Kim[1], Bumjoon J. Kim[1], Hyuk Lee[4], Jihoon Shin[4], Sang Yup Lee[1]

[1]Korea Advanced Institute of Science and Technology (KAIST), Chemical and Biomolecular Engineering, Daejeon, Korea, Rep. of South
[2]Korea Food Research Institute, Korea, Rep. of South
[3]Ewha Womans University, Division of Chemical Engineering and Materials Science, Seoul,
[4]Korea Research Institute of Chemical Technology, Korea, Rep. of South

Background: Aromatic polyesters such as PET are widely used indispensable plastics currently produced from petroleum. Microbial production of polymers from renewable resources has received much attention to substitute the petroleum-based polymers and help solving the environmental problems. Thus, there has been much interest in microbial production of aromatic polyesters from renewable biomass, but without any success.

Objectives: To develop the one-step fermentative production of aromatic polyesters from renewable biomass, the non-natural metabolic pathway for polymerization of various aromatic monomers was established. Then, *Escherichia coli* strain harboring the polymerization pathway was further engineered to efficiently produce aromatic polyesters from glucose.

Methods: The two types of enzymes, CoA-transferases and PHA synthases were examined for the polymerization of aromatic monomers including phenyllactate (PhLA) and mandelate. In addition, the *E. coli* was engineered to overproduce PhLA from glucose based on the *in-silico* genome model simulation.

Results: The metabolic pathway employing *Clostridium difficile* isocaprenoyl-CoA:2-hydroxyisocaproate CoA-transferase and evolved *Pseudomonas* sp. MBEL 6-19 PHA synthase enabled *E. coli* to produce various aromatic polyesters containing PhLA, mandelate, and 3-hydroxy-3-phenylpropionate by feeding the corresponding monomers. In addition, the engineered *E. coli* produced poly(PhLA-*co*-3-hydroxybutyrate) having 11.0 - 84.5 mol% of PhLA from glucose as a sole carbon source. [This work was supported by the Intelligent Synthetic Biology Center through the Global Frontier Project (2011-0031963) and also by the Technology Development Program to Solve Climate Changes on Systems Metabolic Engineering for Biorefineries (NRF2012M1A2A2026556 and NRF-2012M1A2A2026557) from the Ministry of Science and ICT through the National Research Foundation of Korea].

PT032 The marine isolate, Micromonospora aurantiaca strain 30-1: Landlubber or Sea lover?

Marilize Le Roes-Hill[1], Alaric Prins[1], Kim Durrell[1]

[1]Cape Peninsula University of Technology, Biocatalysis and Technical Biology Research Group, Institute of Biomedical and Microbial Biotechnology, Cape Town, South Africa

Background: The marine environment represents a vastly underexplored resource of microorganisms with biotechnological potential. Members of the genus *Micromonospora* can be found in marine environments, but also occur in terrestrial environments, notably plants. Genome sequencing has become an invaluable tool, allowing for insights into the adaptation of these organisms to their respective environments.

Objectives: The objectives of this study were to determine the genome sequence of the marine isolate, *Micromonospora* sp. 30-1; to analyse the genome for genes responsible for a marine and/or terrestrial lifestyle; and to determine its biotechnological potential.

Methods: Genomic DNA was sequenced, assembled using A5 MiSeq and annotated using RAST. Identity of the strain was determined using TrueBac^{TM} ID (EzBiocloud). The genome sequence was analysed for the presence of genes required for survival in marine as well as terrestrial environments. In addition, AntiSMASH and PRISM was used to predict the biosynthetic potential of 30-1. The ability of the strain to produce specific enzymes was determined using standard assays, and bio-activity was determined against a range of pathogens.

Results: Strain 30-1 was identified as *Micromonospora aurantiaca*. Evaluation of the 6.72 Mbp genome sequence indicated the presence of genes that allows for survival in both marine (saline) and terrestrial environments. The strain produces a multicopper oxidase, with optimal production at day 7 in SCN-YE. Production of antibacterial agents were affected by addition of artificial seawater, with activity against Gram positive pathogens being detected. The results indicate a transitioning lifestyle by 30-1, and that it exhibits great biotechnological potential.

PT033 Upcycling of office waste paper and cardbord to biohydrogen

Anna Poladyan[1], Lena Margaryan[2], Karen Trchounian[1], Tatyana Semashko[3], Armen Trchounian[1]

[1]*Yerevan State University, Biochemistry, Microbiology and Biotechnology, Yerevan, Armenia*
[2]*Yerevan State University, Biochemistry, Microbiology and Biotechnology, Yerevan, Armenia*
[3]*Institute of Microbiology, National Academy of Sciences, Laboratory of Enzymes, Minsk, Belarus*

Background: Paper waste (PW) is one among to the mojor components of municipal and inductrial solid wastes which accounts more than 35% of total lignocellosic wastes. Annualy, more than 400 million tons of ligocelluloci PW is generated. PW has potential to serve as renewable feedstoke for biohydrogen production.

Objectives: Growth properties, oxidation reduction potential (ORP) kinetics and hydrogen prodution of *Escherihia coli* parental strain BW25113 and hydrogenase (Hyd) double mutant Δ*hyaB* Δ*hybC* were investiagted upon PW (office waste paper and cardbord) hydrolisate (PWH) utilization.

Methods: PWH was obtained by using dilute acid method in a steam sterilizer for 1 h, 121ºC. Bacteria were grown anaerbically in WPH. pH was measured with pH electrode, ORP and H_2 production – with the platinium and titanium-silicate ones.

Results: The pH of PWH from 1.5 to 7.5 was adjusted by using various alkalis (NaOH, KOH) and potassium buffer; appropriate dilutions of PWH from 1 to 5 were applied and optimal conditions for bacterial growth and H_2 production were designed. Readings of redox Pt electrode dropped up to -500±10 mV, with H_2 yield of ~ 1.45 mmol H_2 L^{-1} at the 4rd h of parental strain growth using PWH with formation of 0.2±0.02 (g bacterial cell dry weight) L^{-1}. Bacterial biomass formation was stimulated ~3-fold upon addition of 0.5% yeast extract and H_2 production was observed at the early beginning of growth log phase. Moreover, mutation of Hyd-1 and Hyd-2 enhanced hydrogen production. Obtained findings are valuable for development of H_2 production biotechnology from wastes.

PT034 Analysis and Control of Mn(II) Ion Leaks During Citric Acid Fermentation by Aspergillus niger

Balázs Fejes[1], Zoltán Németh[1], Ákos Péter Molnár[1], Erzsébet Fekete[2], Áron Soós[2], Béla Kovács[2], Levente Karaffa[1]

[1]*University of Debrecen, Department of Biochemical Engineering, Debrecen, Hungary*
[2]*University of Debrecen, Institute of Food Science, Debrecen, Hungary*

Background: Citric acid (CA; 2-hydroxypropane-1,2,3-tricarboxylic acid) is one of the most important products of industrial biotechnology. Some 2.1 million tons are produced annually and globally by submerged fermentations, employing the fungus *Aspergillus niger*. The process is extremely sensitive to manganese (II) ions; to achieve high CA yields ($Y_{p/s}$), a strongly Mn(II) limited (< 3ppb) growth medium is required. The commonly used stainless steel fermenters are all constructed of similar alloy, where Mn(II) ions are major components.

Objectives: Overtime, the high temperature during sterilization, the acidic pH caused by CA accumulation and the sheer force inflicted by the mechanical agitation will corrode the steel surfaces. This will in turn lead to metal ion – including Mn(II) – leaks into the growth medium, that may severely decrease CA yield.

Methods: To study this phenomenon, lab-scale fermenters were tested. Mn(II) ion concentrations were monitored by ICP-MS.

Results: We showed that sterilization corrupts Mn(II) limitation in the medium when fermenters with damaged steel surface are used. Results suggest that an initial pH of 3.5 that is kept above 2.0 all through the CA fermentation provides the best solution against Mn(II) leaks. Our study indicates that continuous pH control accompanied by regular inspections of the steel surfaces of the fermenter will contribute to achieve high CA yields.

Research was supported by the EU and co-financed by the European Regional Development Fund under the project GINOP-2.3.2-15-2016-00008; by the EFOP-3.6.1-16-2016-00022 project co-financed by the EU and the European Social Fund.

PT035 Screening for cold-active tyrosinases and their application in the bioconversion of resveratrol to piceatannol

Mayowa Agunbiade[1], Zaida Palmer[1], Nuraan Khan[2], Fanie Rautenbach[2], Bronwyn Kirby McCullough[3], Don Cowan[4], Stephanie Burton[5], Marilize Le Roes-Hill[6]

[1]Institute of Biomedical and Microbial Biotechnology, Cape Peninsula University of Technology, Bellville, South Africa, Biocatalysis and Technical Biology Research Group, Capetown, South Africa
[2]Oxidative Stress Research Centre, Faculty of Health and Wellness Sciences, Cape Peninsula University of Technology, Bellville, South Africa, Capetown, South Africa
[3]University of the Western Cape, Bellville, South Africa, Institute for Microbial Biotechnology and Metagenomics, Capetown, South Africa
[4]Centre for Microbial Ecology and Genomics, Department of Genetics, University of Pretoria, Pretoria, South Africa, Pretoria, South Africa
[5]University of Pretoria, Pretoria, South Africa, Vice-Principal: Research and Postgraduate Education, Pretoria, South Africa
[6]Institute of Biomedical and Microbial Biotechnology, Cape Peninsula University of Technology, Bellville, South Africa, Biocatalysis and Technical Biology Research Group, Capetown, South Africa

Background: Tyrosinases are copper-containing monooxygenases known to catalyze two reactions, viz., the *ortho*-hydroxylation of phenols to catechols by means of cresolase activity and subsequent oxidation of catechols to *ortho*-quinones by means of catecholase activity using molecular oxygen. This means that these enzymes can be applied in a wide range of biocatalytic reactions.

Objectives: This study evaluated actinobacterial strains isolated from Antarctic soil samples for the production of tyrosinase, the biochemical characterization of three streptomycete tyrosinases and the potential application of these enzymes in the conversion of resveratrol to piceatannol.

Methods: The enzymes of *Streptomyces* spp. OSM#3, MVTS171#2 and WSML2 were partially purified and biochemically characterized using standard assays for this enzyme group. The partially purified tyrosinases were applied in non-optimized biocatalytic reactions for the conversion of resveratrol to piceatannol.

Results: The enzymes were stable up to 40°C, exhibited variable stability in the presence of different water miscible organic solvents and were able to oxidize L-DOPA, catechol, p-cresol and 4-methylcatechol. Reducing agents revealed an inhibitory effect while typical tyrosinase inhibitors had partial inhibitory effect on the three tyrosinases evaluated in this study. Enzyme activity were greatly enhanced in the presence of Ca^{2+}, Mg^{2+} and Mn^{2+} while, inhibition of enzyme activity was observed in the presence of Co^{2+}, Cu^{2+}, Fe^{2+} and Zn^{2+}. The overall results suggested that WSML2 displayed poor biocatalytic conversion of the substrate resveratrol to the desired product, while OSM#3 and MVTS171#2 were able to catalyze the conversion of resveratrol to piceatannol, thus suggesting their industrial application in biotechnology.

PT036 Genome streamlining in E.coli: a selection scheme for smaller genomes

Edit Tímár[1], György Pósfai[2]

[1]Hungarian Academic of Sciences, Biological Research Centre, Szeged, Institute of Biochemistry, Szeged, Hungary
[2]Hungarian Academic of Sciences, Biological Research Centre, Szeged, Institute of Biochemistry, Szeged, Hungary

Background: Genome reduction projects are motivated by both academic and industrial interests. Streamlining can reveal evolutionary processes shaping bacterial genomes, and reducing complexity and improving metabolic efficiency might aid biotech processes.

Objectives: Current genome reduction efforts are based on targeted approaches. However, rational design of a smaller genome assumes deep, a priori knowledge of gene functions and histories. Moreover, successive targeted deletions allow limited choice of targets and elimination pathways. In contrast, a random, iterative deletion scheme, applied to a large number of cells, and coupled with automatic selection for reduced-genome variants most fit for survival, could provide a straightforward path to streamlined, robust cells.

Methods: Here we propose a selection scheme where smaller genomes provide a growth advantage. The key is to limit the availability of a nucleoside, required for DNA synthesis. To this end, we disabled intrinsic, de novo dTMP synthesis by deleting thyA, and limited the availability of thymidine in the medium.

Results: Using a set of reduced-genome E. coli strains, from wt MG1655 to MDS42, we showed that cells with smaller genomes produced higher yields (higher OD increment) under these conditions. A simple selection scheme for higher yield (repeated cycles of growing individual cells in microwells, followed by mixing, dilution and re-growth), applied to the reduced-genome strains, allowed the isolation of the cells with the smaller genome.

PT037 Microbial production of 1,3-diaminopropane using metabolically engineered Escherichia coli

Tong Un Chae[1], Won Jun Kim[1], Sol Choi[1], Si Jae Park[2], Sang Yup Lee[1]

[1]*Korea Advanced Institute of Science and Technology (KAIST), Chemical & Biomolecular Engineering, Daejeon, Korea, Rep. of South*
[2]*Ewha Woman's University, Chemical Engineering, Seoul, Korea, Rep. of South*

Background: Bio-based production of chemicals from renewable resources is becoming increasingly important for sustainable chemical industry. However, bio-based production of 1,3-diaminopropane (1,3-DAP), C3 diamine, has not yet been demonstrated which has potential to be used as a building block for polyamide, cross linker for epoxy resins, and precursor for pharmaceuticals, agrochemicals and organic chemicals.

Objectives: In this study, *Escherichia coli* was metabolically engineered to produce 1,3-DAP for the first time.

Methods: Comparing heterologous C4 and C5 pathways for 1,3-DAP production by genome-scale *in silico* flux analysis revealed that the C4 pathway employing *Acinetobacter baumannii dat* and *ddc* genes, encoding 2-ketoglutarate 4-aminotransferase and L-2,4-diaminobutanoate decarboxylase, respectively, was the more efficient pathway. In a strain that has feedback resistant aspartokinases, the *ppc* and *aspC* genes were overexpressed to increase flux towards 1,3-DAP synthesis. Also, studies on 128 synthetic small RNAs applied in gene knock-down revealed that knocking out *pfkA* increases 1,3-DAP production. Further overexpression of *ppc* and *aspC* genes were conducted in the *pfkA* deleted strain.

Results: Fed-batch fermentation of the final engineered *E. coli* strain allowed production of 13 g l^{-1} of 1,3-DAP in a glucose minimal medium. [This work was supported by the Technology Development Program to Solve Climate Changes on Systems Metabolic Engineering for Biorefineries from the Ministry of Science, ICT and Future Planning (MSIP) through the National Research Foundation (NRF) of Korea (NRF-2012-C1AAA001-2012M1A2A2026556)]

PT038 Removal characteristics and molecular mechanisms of hexavalent chromium exposed to ochrobactrum sp. cust 210-1

Ying-Chien Chung[1], Chih-Yu Chen[2], Chiu-Yu Cheng[1], Kai-Wei Chou[1], Jong-Tar Kuo[1]

[1]China University of Science and Technology, Department of Biological Science and Technology, Taiwan
[2]Hsing Wu University, Department of Tourism and Leisure, Taiwan

Background: Chromium is extensively used in resistant alloys, electroplating, and leather tanneries. Hexavalent chromium [Cr(VI)] is a toxic and carcinogenic chemical. Bioremediation is an environmentally friendly method of reducing the Cr(VI) concentration and toxicity.

Objectives: *Ochrobactrum* sp. CUST 210-1 is an efficient chromium-reducing bacterial strain that was isolated from the vicinity of an electroplating factory. In this study, the removal characteristics and molecular mechanisms of Cr(VI) by CUST 210-1 were determined to evaluate its feasibility.

Methods: The distribution and fate of Cr(VI) compounds reduced using CUST 210-1 were determined using various analytic techniques. Cr(VI) reductase and a related gene in CUST 210-1 were identified.

Results: The results demonstrated that 3.05% of total Cr was adsorbed on the cell surface in the Cr(VI) form and 8.49% was adsorbed in the Cr(III) form. The soluble Cr(III) complex was present as $Cr(OH)_4^-$, $Cr_2O_2(OH)_4^{2-}$, or $Cr_3O_4(OH)_4^{3-}$. The Cr precipitate was present as $Cr(OH)_3$ and accounted for 84.71% of total Cr. These results were consistent with the results of SEM, EDS, TEM, SAED, FTIR, XRD, and UV-Vis spectroscopy analyses. CUST 210-1 possesses two types of chromate reductase: ChrR and CrS. Through genetic analysis, the main local genomic context of *chrR* genes was determined to contain a group of *chrB*, *chrA*, and *chrC* genes. The *chrR* gene from CUST 210-1 exhibited 99.2% similarity with that from *Escherichia coli*, and 92.6% similarity with that from *Gluconacetobacter* sp., as determined through BLASTN analysis. ChrR found in CUST 210-1 was 94.8% homologous with that found in *Gluconacetobacter* sp.

PT039 Whole genome analysis of Aspergillus sojae SMF 134 supports its merits as a starter for soybean fermentation

Inhyung Lee[1], Kang Uk Kim[1], Kyung Min Kim[1], Yong-Ho Choi[2], Byung-Serk Hurh[2]

[1]Kookmin University, Bio and Fermentation Convergence Technology, BK21+ Project, Seoul, Korea, Rep. of South
[2]Sempio Foods Company, Sempio Fermentation Research Center, Chungcheongbukdo, Korea, Rep. of South

Background: *Aspergillus sojae* is a *koji* (starter) mold that has been applied for food fermentation in Asia. Recently, we have isolated the *A. sojae* SMF 134 strain from *meju* (Korean soybean fermented brick) which has great potential to be developed as a starter for soybean fermentation because of its high protease and leucine aminopeptidase (LAP) activities.

Objectives: In this study, the whole genome of SMF 134 was analyzed to evaluate its potential as a starter for soybean fermentation at the genomic level.

Methods: Genome sequencing was performed using the PacBio RS system and the genome of *A. sojae* SMF 134 was analyzed using seveal programs available through web sites.

Results: The genome size was 40.1 Mbp, which is predicted to be composed of 8 chromosomes with 13,748 ORFs. SMF 134 had a total of 151 proteases, and the number of total proteases, especially aminopeptidases and carboxypeptidases, was more than *A. oryzae*. RNA seq analysis revealed that some endopeptidase genes were highly expressed during soybean fermentation, suggesting that SMF 134 efficiently degrades soybean proteins to small peptides. In addition, two more *lap* genes were found in addition to the previously known *lap1* in *A. sojae* and these three *lap* genes were confirmed to be expressed. Three γ-glutamyltranspeptidase (*ggt*) genes were also identified and higher GGT activity was observed in SMF 134 than in RIB 40. Secondary metabolite gene cluster analysis confirmed that SMF 134 is neither an aflatoxin nor cyclopiazonic acid (CPA) producer like other *A. sojae*.

PT040 Expression of recombinant hyaluronidase from Talaromyces stipitatus in Pichia Pastoris model system

Lenka Bobková[1], Jana Jílková[1], Sofia Chatzigeorgiou[1;2], Eva Procházková[1], Matouš Čihák[1;2], Zbyněk Černý[1], Vladimír Velebný[1]

[1]Contipro a.s., Dolní Dobrouč, Czech Republic
[2]First Faculty of Medicine, Charles University, Institute of Immunology and Microbiology, Prague, Czech Republic

Background: Hyaluronidases are enzymes that degrade hyaluronic acid, decreasing molecular weight of this polysaccharide. Final products of such reaction are typically oligosaccharides consisting of low number of monosaccharide units. Hyaluronidases are well known in the animal kingdom, their presence among vertebrata, mollusca and insects have already been documented. On the contrary, there is only a limited number of reports which describe these enzymes in fungi.

Objectives: In the presented study we report the expression of a recombinant hyaluronidase from *Talaromyces stipitatus*. Following the discovery of hyaluronidase activity in this fungal species (Bobková et. al. 2018), gene coding for the enzyme was selected from nucleotide database based on sequence similarity to other hyaluronidases.

Methods: The coding sequence was synthetized and transformed into *Pichia pastoris* strain. We were able to express active recombinant enzyme and purify it by ion exchange chromatography. Hyaluronidase activity was verified by different methods. For screening purposes, hyaluronidase activity was detected using petri dishes with hyaluronic acid. The products of the enzymatic reaction were identified by LC-MS method and the method for specific enzymatic activity determination was based on decrease in viscosity of the substrate.

Results: The products of the enzymatic reaction were saturated oligosaccharides with N-acetylglucosamine on their reducing ends. The products are similar to those of mammalian hyaluronidases, indicating similar mechanism of the reaction.

PT041 Towards Systems Biology to study bioconversion of hydrocarbons and metals in Rhodococcus

Martina Cappelletti[1], Alessandro Presentato[2], Andrea Firrincieli[3], Raymond J. Turner[2], Davide Zannoni[1]

[1]*University of Bologna, Department of Pharmacy and Biotechnology, Bologna, Italy*
[2]*University of Calgary, Department of Biological Sciences, Calgary, Canada*
[3]*University of Tuscia, 1Department for the Innovation in Biological Systems, Agro-Food and Forestry, Viterbo, Italy*

Background: Members of *Rhodococcus* genus, which are widely distributed in terrestrial and marine environments, show outstanding metabolic diversity and flexibility deriving from their capacity to use different organic (toxic) contaminants by converting them into high value-added products, e.g. triacylglycerol. Rhodococci also withstand various stress conditions as they grow in the presence of high-concentrations of solvents and metal(lloid)s. These peculiar properties make *Rhodococcus* spp. as promising candidates for industrial applications. In this respect, systems biology studies can provide integrated views to elucidate stress response and regulation mechanisms of bio-degradation/conversion pathways.

Objectives: We provide a combination of functional and molecular data on the response of *R. aetherivorans* BCP1 cells to hydrocarbon contaminants and toxic metalloids. These results establish the bases for the development of systems biology approaches aimed at optimizing *Rhodococcus* strains performances in bioremediation applications related to metal- and/or oil- recovery.

Methods: Phenomic, metabolomic, and transcriptomic approaches were combined with molecular and physiological analyses of wild type and mutant cells to study the response of *R. aetherivorans* BCP1 grown on different hydrocarbons, i.e. naphthenic acids, alkanes, and/or in the presence of toxic metalloids, i.e. tellurium, selenium and arsenic.

Results: *R. aetherivorans* BCP1 cells convert different hydrocarbons into neutral lipids while they reduce toxic metalloid oxyanions into metal nanostructures. Integrated omic data on the genetic determinants and regulatory mechanisms involved in these microbial processes are shown. These combined molecular and functional results implement our present capacity of modeling, predicting and optimizing the use of *Rhodococcus* strains in industrial applications.

PT042 Identification, expression and biochemical characterization of AMP phosphorylases in extreme environments

Noha Attallah[1;2], Sarah Kamel[2], Maryke Fehlau[2;3], Rania Siam[1;4], Peter Neubauer[2], Anke Wagner[2;3]

[1]*The American University in Cairo, Biotechnology Graduate program, School of Sciences and Engineering. , Cairo, Egypt*
[2]*Technische Universität Berlin, Institute of Biotechnology, Department of Bioprocess Engineering, Berlin, Germany*
[3]*BioNukleo GmbH, Berlin, Germany*
[4]*The American University in Cairo, Yousef Jameel Science and Technology Research Center, Cairo, Egypt*

Background: Nucleotide analogues are interesting pharmaceutical intermediates as they represent the active form of nucleoside analogue drugs that are used in treatment of cancer or viral infections. Furthermore, they are used as precursors in preparation of artificial oligonucleotides for therapeutic or diagnostic use.

Enzymes as active biocatalysts offer numerous advantages over traditional chemical processes with respect to high process selectivity and efficiency. Recently, adenosine-5'-monophosphate phosphorylase (AMP-P) was identified to catalyze the reversible phosphorolysis of nucleotides into nucleobase and ribose-1,5-bisphosphate. Therefore, it is an attractive and promising biocatalyst in synthesis of nucleotides and their analogues.

Objectives: The availability of enzymes with wide substrate spectrum is an important prerequisite to produce a variety of modified nucleotides in enzymatic processes. Therefore, interesting AMP-Ps were produced and characterized concerning their substrate spectra.

Methods: Red sea metagenomic data and sequences of the National Center for Biotechnology Information database were screened for putative AMP-Ps. Phylogenetic analysis was performed to identify interesting candidates based on their phylogenetic distance. AMP-Ps were expressed in *E. coli*, purified and characterized (substrate spectrum, pH and temperature optimum)**.**

Results: AMP-Ps were chosen from different phylogenetic clusters for gene synthesis based on difference in the active residues and variability of their isolation extreme environment. A set of AMP-Ps was successfully expressed in *E. coli*. Expression conditions were optimized to reach higher amounts of soluble protein. Activity assays revealed NMP phosphorolytic activity of the tested enzymes. In this study, we confirmed the hypothesis that extreme environments can also provide cytoplasmic enzymes with novel characteristics.

PT043 Engineering natural products: the abyssomicins

Alba Iglesias Vilches[1], Jem Stach[1]

[1]*School of Natural and Environmental Sciences, Newcastle upon Tyne, United Kingdom*

Background: The tetronate family of natural products constitutes a structurally and functionally diverse group of secondary metabolites whose potent bioactivities (antimicrobials, antivirals, and anti-cancer agents) makes them attractive targets for clinical and industrial exploitation. Abyssomicin C, produced by the marine actinomycete Verrucosispora maris AB-18-032, is a type I polyketide antibiotic active against Gram-positive bacteria including methicillin-resistant *Staphylococcus aureus* (MRSA). It is the first natural product that inhibits the formation of *p*-aminobenzoic acid, a constituent of the folate pathway.

Objectives: Abyssomicin biosynthesis is highly amenable to re-engineering, as the enzymes involved in the synthesis of the tetronate (AbyA1) and the spiro-tetronate-forming Diels-Alderase (AbyU) are both capable of accepting structurally diverse substrates. The aim of this project is to assemble synthetic operons, based on the wild-type abyssomicin pathway, that will produce diverse polyketides, and thence, novel tetronate compounds for biopharmaceutical development.

Methods: For that, the native biosynthetic gene cluster was cloned into four different chassis: *E. coli* BAP1, *S. albus* J1074, *S. lividans* K4-114 and *S. coelicolor* M145. These chassis were engineered via CRISPR-Cas9 and abyssomicin C production was monitored by HPLC/MS.

Results: With an heterologous host capable of producing abyssomicin C, future work will focus on refactoring the abyssomicin gene cluster, to produce polyketides of various lengths and substitution/oxidation patterns. This will be primarily achieved through the production of plug-and-play operons and heterologous promoters in both the wild-type producer and heterologous hosts.

PT044 Disruption of the gene TMI1 improves the efficiency of ethanol production from xylose and glucose in Scheffersomyces stipitis

Marta Semkiv[1], Mariia Borbuliak[1], Orest Hryniv[1], Krzysztof Berezka[2], Tomas Linder[3], Kostyantyn Dmytruk[1], Volkmar Passoth[3], Andriy Sibirny[1;2]

[1]*Institute of Cell Biology of NAS of Ukraine, Department of Molecular Genetics and Biotechnology, Lviv, Ukraine*
[2]*University of Rzeszow, Department of Biotechnology and Microbiology, Rzeszow, Poland*
[3]*Swedish University of Agricultural Sciences, Department of Microbiology, Uppsala, Sweden*

Background: Lignocellulosic biomass represents an abundant renewable energy source and it is considered an ideal substrate for fuel ethanol production. However, the feasible technology for the bioethanol production from lignocellulosic materials has not been developed yet. The main reason is the absence of a robust microorganism capable of efficient alcoholic fermentation of the main sugars of lignocellulose, most importantly, glucose and xylose. Yeast *Scheffersomyces stipitis* is able to ferment both glucose and xylose with a high ethanol yield.

Objectives: We aimed to identify potential effectors of fermentation in *S. stipitis* by the insertional mutagenesis combined with the positive selection of ethanol overproducers and to investigate the role of identified gene.

Methods: Inhibitor of key glycolytic enzymes 3-bromopyruvate was used as a selection agent for obtaining of ethanol overproducers.

Results: Among the selected 3-bromopyruvate resistant mutants, strain #4.6 revealed reproducible increase of ethanol accumulation during xylose and glucose fermentation. In this strain, the insertion was found within the ORF of a gene homologous to *Saccharomyces cerevisiae* gene *YDL119C*, encoding mitochondrial transporter. We designated identified gene *TMI1*. Wild-type phenotype was restored via complementation of the insertional mutation by the wild type allele of *TMI1* gene, and the deletion of *TMI1* gene on the background of *Ku80* strain improved its ethanol production on glucose/xylose containing media. The respiration efficiency and key glycolytic enzymes activities were measured in all investigated strains. Obtained results revealed that the gene *TMI1* is involved in the regulation of alcoholic fermentation of glucose and xylose in *S. stipitis.*

PT045 Biosynthesis of single- and multi-element nanomaterials using recombinant Escherichia coli

Kyeong Rok Choi[1], Yoojin Choi[1], Tae Jung Park[2], Doh C. Lee[1], Sang Yup Lee[1]

[1]Korea Advanced Institute of Science and Technology, Department of Chemical and Biomolecular Engineering, Daejeon, Korea, Rep. of South
[2]Chung-Ang University, Department of Chemistry, Seoul, Korea, Rep. of South

Background: Biosynthesis of nanomaterials (NMs) are attracting attention. However, principles underlying the producibility of crystalline/amorphous NMs in biological systems has not been understood yet.

Objectives: We identify critical factors determining the producibility of NMs in biological systems and synthesize various NMs including those that have not been synthesized so far.

Methods: A recombinant *Escherichia coli* strain co-expressing metallothionein and phytochelatin synthase and its lysate were used for *in vivo* and *in vitro* biosynthesis of NMs. In addition, 35 candidate elements suitable as precursor of NM biosynthesis were screened from the periodic table. Pourbaix diagram was also screened to identify appropriate pH and reduction potential conditions stably maintaining crystal phase of chemicals species composed of either of the 35 candidate elements or their oxide/hydroxide forms.

Results: Among the 35 elements screened, crystalline NMs of nine elements (Mn_3O_4, Fe_3O_4, Cu_2O, Mo, Ag, $In(OH)_3$, SnO_2, Te, and Au) are successfully biosynthesized while amorphous or no NMs are syntheized for the other 16 elements. Biosynthesis of crystalline NMs, however, can be achieved by modifying pH condition based on Proubaix diagram. Moreover, crystalline NMs with multple elements ($CoFe_2O_4$, $NiFe_2O_4$, $ZnMn_2O_4$, $ZnFe_2O_4$, Ag_2S, Ag_2TeO_3, Ag_2WO_4, Hg_3TeO_6, $PbMoO_4$, $PbWO_4$, and $Pb_5(VO_4)_3OH$) are sucessfully biosynthesized using the strategy. This report will facilitate biosynthesis of NMs with valuable properties in an environment-friendly manner. [This work was supported by the Technology Development Program to Solve Climate Changes on Systems Metabolic Engineering for Biorefineries (NRF-2012M1A2A2026556 and NRF-2012M1A2A2026557) from the Ministry of Science and ICT through the National Research Foundation of Korea.]

PT046 Systems Biology approach to identify genes of influence in Carbapenem Resistant Enterobacteriaceae gene network

Somorita Baishya[1], Anupam Das Talukdar[2], Anand Anbarasu[3], Manabendra Dutta Choudhury[1]

[1]*Assam University, Life Science and Bioinformatics, Silchar, India*
[2]*ASSAM UNIVERSITY, Life Science and Bioinformatics, Silchar, India*
[3]*VIT University, Division of Bioinformatics, Vellore, India*

Background: Carbapenem Resistance is an alarming situation because it is the last resort for treatment of severe Gram-negative infections. This resistance is mainly acquired due to the transfer of carbapenemase encoding genes like NDM. Since, NDMs are associated with dissemination of the resistance therefore it would be interesting to study the influential genes of carbapenem resistance and also to check the susceptibility pattern in absence of the influential genes. This difference in susceptibility pattern will help in concluding the role of those genes in imparting resistance and thus help in identifying newer targets for designing antibiotics.

Objectives: To find out influential genes in the network from in silico analysis and confirm their role by phenotypic and genotypic analysis

Methods: For in silico analysis, genes associated with carbapenem resistance owing to NDM genes are retrieved from NCBI Database. STRING database was used to check protein- protein interaction. MCODE and Network Analyzer plugin of Cytoscape software were used to find out the influential genes of the network. DAVID database was used to verify their functional enrichment roles. PCR was done for the selected genes under the influence of different carbapenems to confirm their presence. Since the genes were plasmid mediated, therefore, SDS was used to cure the plasmid. Elimination of the plasmids was confirmed by antibiotic susceptibility testing and molecular characterization.

Results: *RepA* gene was found to be influential from in silico analysis. *RepA* is associated with plasmid DNA replication. With the elimination of *repA* gene *E.coli* became susceptible to different carbapenems.

PT047 Structural characterisation of Hsf: A Trimeric Autotransporter Adhesin

Anchal Malik[1], Adrian Goldman[2]

[1]*University of Leeds, Faculty of biological sciences, Leeds, United Kingdom*
[1]*University of Leeds, Faculty of biological sciences, Leeds, United Kingdom*

Background: Out of all *Haemophilus influenzae,* type b (Hib) is the most virulent serotype, that can lead to invasive conditions like bacteraemia, pneumonia and meningitis. One of the important virulent factors of Hib is Haemophilus surface fibril (Hsf). Hsf belongs to trimeric autotransporter adhesin (TAA), type V subfamily of secreted proteins. Binding of Hsf to vitronectin, a complement inhibitor, found in both serum and extracellular matrix allows it to evade the host immune system. Hence, marks its importance as potential drug target.

Hsf shares 72% of sequence identity with Hia, a well-characterized TAA of *Haemophilus influenzae* in their shared region of binding domains 1 and 2. The size difference of trimeric Hsf (~750 kDa) and Hia (~340 kDa) is due to the third binding domain and three putative domains with unknown functions in Hsf. Early EM studies suggest that Hsf forms a novel hairpin like architecture, unlike other TAAs.

Objectives: Following the hypothesis that hairpin loop architecture of Hsf may contribute to its adhesion, we aim to solve the structure of Hsf and of its six domains by cryo-EM and X-ray crystallography.

Methods:

- Soluble and membrane protein purification to achieve >95% pure protein.
- SEC-MALLS and MS for interdomain and protein: serum interactions.
- X-ray crystallography and cryo-EM for structural characterization.

Results: All Hsf domains were cloned, overexpressed and purified to >95% purity. Crystallization hits were obtained in 96-well matrix. The hits were optimized in 24-well matrix, followed by diffraction data collection.

PT048 On-fiber display of a functional peptide at sites distant from the cell surface using a trimeric autotransporter adhesin

Katsutoshi Hori[1], Hajime Nakatani[1], Junichi Kanie[1]

[1]*Nagoya University, Dept. Biomolecular Engineering, Nagoya, Japan*

Background: Displaying peptides or proteins on the cell surface is considered to be a remarkable technology to solve common problems in bioprocesses using microorganisms because of the low reaction efficiency due to the mass transfer limitations. The distance from the cell surface to those functional molecules should be an important factor because the function of a bacterial surface protein is interfered with other cell surface structures, such as pili and LPS.

Objectives: This study aimed to develop an on-fiber display system of a functional peptide using AtaA from *Acinetobacter* sp. Tol 5, a long trimeric autotransporter adhesin fiber, which can vary the distance between the displayed molecule and the cell surface.

Methods: We constructed His-tagged full length and shorter AtaA fibers designed by N-terminal deletion and expressed them in Tol 5 *ΔataA* mutant and *Escherichia coli* cells. The distance from the cell surface to the His-tag was measured using immuno-electron microscopy. The functionality of the His-tag on the AtaA derivative fibers was examined by Ni-Sepharose beads using CLSM and the fluorescence intensity of the cells adhering to the beads. The cells on the beads were also quantified by measuring cell bound enzymatic activities.

Results: The His-tag was displayed on the fiber tip located at fixed distances from the cell surface. N-terminal deletion of AtaA shortened the distance between the His-tag and the cell surface, as designed. Time-course analyses of the cell-to-Ni-Sepharose beads binding revealed that cells producing the longer fibers bound more rapidly to the beads.

PT049 *In Vivo* Thrombolytic Activities of Streptokinase from *Streptococci* species with the k-Carrageenan Induced Rat Tail Thrombosis Model

Sarita A. Bhutada[1]

[1]*Department of Microbiology, Sanjivani Arts, Commerce and Science College, Kopargaon, (INDIA)*

Backgrounds: Streptokinase is a very well known novel fibrinolytic protein. It is produced by several species of Streptococci and possesses therapeutic importance. Thrombosis is one of the most widely occurring diseases in modern life. Streptokinase is considered effective drugs for thrombolytic therapy.

Objectives: The objective of this study was to demonstrate the activity of Streptokinase from *Streptococci* species *in vitro* and *in vivo*.

Method: In the present study soil samples were collected from nearby ENT hospital. Isolated *Streptococci* strains showed β-haemolysis and subjected to caseinolysis assay and blood clot dissolving assay. *In vivo* thrombolysis efficiency of Streptokinase, a nontoxic fibrinolytic enzyme purified from *Streptococci* species, was affirmed by significant inhibition of thrombus formation in the k-carrageenan-induced in wistar rats tail, in a dose-dependent manner.

Results: The optimum temperature and pH of Streptokinase is 27-37°C and 9 respectively. The molecular weight of Streptokinase was obtained 47kDa. The thrombus observed in the crude enzyme treatment and histological examination confirmed thrombosis. Streptokinase enzyme produced by *Streptococci* species found capable of degrading thrombus induced in Wistar rats. These findings unequivocally suggest that Streptokinase may serve a promising alternative to the commercial thrombolytic drugs

PT050 Actinobacteria associated with two diverse soil environments and their multicopper oxidase diversity

Alaric Prins[1;2], Bronwyn Kirby-McCullough[1], Marilize Le Roes-Hill[2]

[1]Institute of Microbial Biotechnology and Metagenomics, University of the Western Cape, Department of Biotechnology, Cape Town, South Africa
[2]Biocatalysis and Technical Biology, Institute of Biomedical and Microbial Biotechnology, Cape Peninsula University of Technology, Cape Town, South Africa

Background: Multicopper oxidases (MCOs) are a family of enzymes that includes bilirubin oxidases, ceruloplasmin and laccases. The phylum Actinobacteria is one of the largest and most diverse groups within the domain Bacteria and recent genome studies have shown the presence of MCO genes in actinobacteria, and therefore, these organisms may serve as a potential source of novel MCOs.

Objectives: The objectives of this study were: 1) to determine the actinobacterial diversity associated with soil from two diverse environments; 2) to identify unique MCOs through culture and culture-independent methods; and 3) to clone, heterologously express and biochemically characterise the two new MCOs.

Methods: Soil was sampled from two environments – nutrient-poor soil associated with *Aspalathus linearis*, and organic-rich soil from a developing peatland. Actinobacteria was isolated from both environments and screened for MCO activity. Metagenomic DNA extracted from the soils was sequenced using actinobacterial-specific 16S rRNA primers. Two putative MCO genes, SF1.4MCO1 and SF1.4MCO2, that was identified from one of the isolates (SF1.4) were cloned into a pET and HaloTag® expression system, respectively.

Results: A large percentage of the actinobacterial isolates from the organic-rich soil showed extracellular laccase activity in liquid screen studies. Primary analyses of the sequencing data revealed a high actinobacterial diversity in both soil types. Two MCO genes identified from an actinobacterial isolate, SF1.4, showed homology to two bilirubin oxidases in the Laccase and Multicopper Oxidases Engineering Database (LccED). Preliminary expression studies showed good levels of expression. Biochemical characterisation of the two MCOs will be performed in future studies.

PT051 Microinjection of blue fluorescent protein into bacterial giant protoplasts

Sawako Takahashi[1], Hiromi Nishida[1]

[1]*Toyama prefectural University, Biotechnology, Imizu, Japan*

Background: Generally, a cell diameter greater than 20 μm, is required for microinjection. Thus, this technique is not applicable to bacterial cells unless their size is increased. Bacterial protoplasts and spheroplasts can enlarge in the presence of metal ions and an inhibitor of peptidoglycan synthesis under suitable osmoprotective condition. We selected the gram-negative bacterium *Lelliottia amnigena* for microinjection from enlarged protoplasts or spheroplasts of different bacteria, for example, *Bacillus*, *Deinococcus*, *Escherichia*, and others. However, cytoplasmic microinjection was not successful because of the unsuitable incubation medium.

Objectives: We considered the metal salt composition, especially Ca^{2+} and Mg^{2+}, in the incubation medium and established a method for generating bacterial cells capable of microinjection of DNA, RNA and protein.

Methods: *L. amnigena* was incubated in the medium with different concentrations of metal salts, containing penicillin G. The *L. amnigena* enlarged spheroplast culture was mounted on a glass slide, and placed on the microscope stage. The microinjection needle was inserted into the spheroplast, while releasing BFP solution.

Results: The optimal incubation conditions were identified and utilized to generate enlarged spheroplasts suitable for microinjection. We tried to pierce the microinjection-needle into 9 cells. The microinjection-needle was pieced into all 9 cells, indicating that Ca^{2+} and Mg^{2+} generated the cells with the suitable flexibility and viscosity for microinjection. Although six of the nine cells collapsed during microinjection, BFP solution was successfully microinjected into the cytoplasm of three cells, which were confirmed through fluorescent microscopy. This is the first achievement of microinjection technique to bacterial cells.

PT052 Tuning the transcription and translation of L-amino acid deaminase in Escherichia coli improves a-ketoisocaproate production from L-leucine

Yang Song[1], Xuping Shentu[1], Xiaoping Yu[1]

[1]Zhejiang Provincial Key Laboratory of Biometrology and Inspection & Quarantine, College of Life Sciences, China Jiliang University, Hangzhou, China

Background: α-Ketoisocaproate (KIC) is used widely in the pharmaceutical and nutraceutical industries. In previous studies, we achieved a one-step biosynthesis of KIC from L-leucine, using an *Escherichia coli* whole-cell biocatalyst expressing an L-amino acid deaminase (L-AAD) from *Proteus vulgaris*. However, the KIC production was limited by the low L-AAD expression.

Objectives: To improve the α-ketoisocaproate production by tuning the transcription and translation of L-amino acid deaminase in *Escherichia coli*.

Methods: The N-terminal codon of L-AAD in the downstream region was initially redesigned, and then the ribosome-binding site (RBS) sequences were modulated to improve translation efficiency. In addition, the plasmid origin was optimized to offer various amounts of the DNA template for transcription. Finally, these three strategies were combined to improve the expression levels of L-AAD.

Results: By optimizing the plasmid origin with different copy numbers, modulating messenger RNA structure downstream of the initiation codon, and designing the sequences at the ribosome binding site, we increased biocatalyst activity to 31.77%, 24.89%, and 30.20%, respectively, above that achieved with BL21/pet28a-lad. The highest KIC titers reached 76.47 $g \cdot L^{-1}$, 80.29 $g \cdot L^{-1}$, and 81.41 $g \cdot L^{-1}$, respectively. Additionally, the integration of these three engineering strategies achieved an even higher KIC production of 86.55 $g \cdot L^{-1}$ and a higher L-leucine conversion rate of 94.25%. Therefore, design at the transcriptional and translational levels effectively improves biocatalyst activity and KIC titers. These transcriptional and translational regulation strategies proposed herein may be generally applicable to the construction of other biocatalysts.

PT053 Calcium ion-dependent enlargement and fusion of Deinococcus grandis spheroplasts

Koki Nishino[1], Rintaro Tsuchikado[1], Hiromi Nishida[1]

[1]*Toyama Prefectural University, Department of Biotechnology, Imizu, Japan*

Background: Bacterial spheroplasts enlarge by incubation in the presence of an inhibitor of peptidoglycan synthesis under osmotically protective condisitons. Enlargement of spheroplasts of *Deinococcus grandis*, an aerobic Gram-negative, non-spore-forming, radioresistant, rod-shaped bacterium, requires calcium or magnesium ion (1).

Objectives: In this study, we determined the effects of different concentrations of these divalent cations on *D. grandis* spheroplast enlargement.

Methods: Lysozyme-treated spheroplasts were centrifuged and resuspended in the medium which contains 5 g/L peptone, 1 g/L yeast extract, and 0.1 g/L ferric citrate with different concentrations of metal salts, containing 300 μg/mL penicillin G. The spheroplasts were incubated at 24◦C.

Results: We compared the cell sizes of the spheroplasts at five different concentrations of calcium or magnesium ion (2). At 16.2 mM of calcium or magnesium ion, the inner membrane of *D. grandis* spheroplasts collapsed and the spheroplasts did not enlarge. At 333 mM of calcium or magnesium ion, enlargement was inhibited. At 200 mM of calcium ion, the outer membranes of *D. grandis* spheroplasts were fused repeatedly, but the inner membranes were not fused. Thus, at 200 mM of calcium ion, giant cells that have multiple cytoplasms were observed and were ≥ 500 μm in diameter. However, cell fusions were not observed in any concentrations of magnesium ion. Our findings show calcium and magnesium ions have different effects on the outer membrane synthesis and structure.

(1) Nishino K, et al. (2018) Microbiology 164, 1361-1371. (2) Nishino K, Nishida H (2019) FEMS Microbiology Letters 366, in press.

PT054 Reconstructing Biosynthetic Pathway of Plant-derived Cancer Chemopreventive-Precursor Glucoraphanin in E. coli

Bo Yu[1]

[1]*Institute of Microbiology, Chinese Academy of Sciences, CAS Key Laboratory of Microbial Physiological and Metabolic Engineering, Beijing, China*

Background: Epidemiological data confirmed a strong correlation between regular consumption of cruciferous vegetables and lower cancer risk. This cancer preventive property is mainly attributed to the glucosinolate products, such as glucoraphanin found in broccoli that is derived from methionine.

Objectives: Low amounts in plant tissues and high cost of extraction have limited the production of glucoraphanin. Metabolic engineering in heterologous organisms is an attractive approach to achieve efficient production of valuable natural products. In this study, we attempt to produce glucoraphanin in engineered E. coli strain.

Methods: We used branched-chain amino transferase 3 to catalyze two transamination steps to ensure the purity of precursor molecules and used cysteine as a sulfur donor to simplify the synthesis pathway. Two chimeric cytochrome P450 enzymes were engineered and expressed in E. coli functionally. The original plant C-S lyase was replaced by the *Neurospora crassa* hercynylcysteine sulfoxide lyase. Other pathway enzymes were successfully mined from *Arabidopsis thaliana*, *Brassica rapa*, and *Brassica oleracea*.

Results: We report the first successful reconstruction of the complete biosynthetic pathway of glucoraphanin from methionine in Escherichia coli via gene selection, pathway design, and protein engineering. Biosynthesis of glucoraphanin upon co-expression of the optimized enzymes in vivo was confirmed by liquid chromatography-tandem mass spectrometry analysis. No other glucosinolate analogs (except for glucoiberin) were identified that could facilitate the downstream purification processes. Production of glucoraphanin in this study laid the foundation for microbial production of such health-beneficial glucosinolates in a large-scale.

PT055 Mechanisms of micropollutant degradation by Schizophyllum commune

Brigit van Brenk[1]

[1]*Utrecht University, Biology, Utrecht, Netherlands*

Background: Water dissolved poly-aromatic hydrocarbons, like herbicides, pharmaceuticals and textile dyes, can have a high risk for the environment. Mushroom forming fungi are able to degrade such compounds but the underlying mechanisms are largely unknown. This hampers strain optimization for waste water treatment.

Objectives: The aim of this study is to identify genes involved in poly-aromatic hydrocarbon degradation with the aim to improve strains to be used in waste water treatment.

Methods: *Schizophyllum commune* is a model organism for mushroom forming fungi. It completes its life cycle in 10 days, can be grown in small scale such as microtiter plates, and can be genetically modified. Notably, genome sequences are available of a variety of strains showing an overall genetic diversity of 15%. We have a collection of 100 *Schizophyllum commune* strains. These strains were screened for degrading activity of six different dyes, like remazol brilliant blue R, orang G , and malachite green.

Results: A huge variation was observed in polyaromatic degrading activity of the different strains of *S. commune*. Some strains were unable to degrade dyes while other readily removed the dye from the culture medium. We will now cross these strains to perform a bulked segregant analysis with the aim to identify the genes responsible for micropollutant degradation.

PT056 Large-scale cell-free production and purification of the Plasmodium falciparum Chloroquine Resistance Transporter (PfCRT)

Lea Madlen Petersen[1], Eric Beitz[1]

[1]*University of Kiel, Dept. of Pharmaceutical and Medicinal Chemistry, Kiel, Germany*

Background: Heterologous expression is an important, routinely used tool for functional and structural characterization of proteins of medicinal and pharmaceutical relevance, which are usually present at too low concentrations in their natural host. However, overexpression of membrane proteins still remains a challenging task due to their potential toxicity to the host organism, and folding and solubility issues.

Objectives: We aimed at expressing the integral membrane protein PfCRT, which plays a major role in conveying resistance against antimalarial drug chloroquine, using a cell-free system, and to subsequently purify the protein for determination of its yet unknown structure and for functional studies.

Methods: Fermentation of *E. coli,* subsequent cell disruption, and stepwise ultracentrifugation was carried out to obtain an S30 ribosome extract. PfCRT was then cell-free produced using a detergent-based approach. Correct folding was evaluated using the green fluorescent protein as folding indicator. Immobilized metal affinity chromatography and size-exclusion chromatography were performed to obtain purified PfCRT.

Results: We successfully expressed PfCRT in the cell-free system with Brij78 proving to be a suitable detergent for direct translation into micelles. Up to 1.5 mg PfCRT protein per ml reaction mixture were obtained, appearing as partially SDS-resistant oligomers. We examined different detergents for purification. Of these, DDM, Foscholine-12, Brij78 and LMNG were the most promising detergents. In conclusion, cell-free synthesis proved to be a rapid method for obtaining high concentrations of purified PfCRT for prospective structural and functional studies.

PT057 Green-synthesis of tellurium nanoparticles by azoarcus sp cib

Elena Alonso[1], Eduardo Díaz[1], Manuel Carmona[1], Laura Castro Ruiz[2]

[1]Biological Research Center, Microbial and Plant Biotechnology Department, Madrid, Spain
[2]Complutense University of Madrid, Department of Materials Science and Metallurgical Engineering, Faculty of Chemical Sciences, Madrid, Spain

Background: The use of metallic nanoparticles (MeNPs) has increased exponentially due to the great technological development. Bioproduction of MeNPs is a green alternative to the chemical synthesis. Some bacteria allow to couple the removal of contaminant metals from the environment to the production of MeNPs. *Azoarcus* sp. CIB, a beta-proteobacterium that shows resistance to certain metals and metalloids, was reported to produce SeNPs. Here, we explore further *Azoarcus* sp. CIB as a suitable biocatalyst to produce other MeNPs.

Objectives: Analysis of the resistance of *Azoarcus* sp. CIB to tellurite and study of the molecular mechanisms involved. Exploring the ability of strain CIB to produce tellurium nanoparticles (TeNPs) and search for the more suitable bioproduction conditions.

Methods: The toxic effect of Te was evaluated by monitoring intracellular reactive oxygen species (ROS) with the fluorescent H2DCFDA probe and thiol oxidation. Bioproduction of TeNPs was evaluated in growing cultures and resting cell conditions, and TeNPs produced were analyzed by TEM.

Results: *Azoarcus* sp. CIB cells grown in minimal medium were able to resist 1 mM tellurite. The products of the genes *tpmt* (SAM-methyltransferase) and *arsB* (arsenite extrusion pump) might be involved in tellurite resistance. In CIB strain tellurite is able to produce protein damage in thiol groups, but intracellular ROS is lower than that obtained with other strong oxidants. We have established an *Azoarcus* sp. CIB-based process to produce intracellular rod-shaped TeNPs with an average length of 218 ± 165 nm, that can increase to nanocable size when long-time incubations were performed.

PT058 Analyzing the potential of the African elephant gut microbiome for biogas optimization

Elzette van der Walt[1], Errol Duncan Cason[1], Jan-G Vermeulen[1], Angel Valverde[1], Marcele Vermeulen[1]

[1]*University of the Free State, Microbial, biochemical and food biotechnology, Bloemfontein, South Africa*

Background: The production of biogas via anaerobic digestion has attracted attention not only as a renewable green energy source, but a cost-effective waste management strategy. However, the degradation of low-yield substrates high in fiber (e.g., wood and crop residues) pose a particular challenge. The African elephant (*Loxodonta africana*) maintains its body weight on a diet supplemented with fiber and lignin rich compounds like tree bark, branches and roots. This ability may be attributed to their gut microbiome, which has evolved to extract energy and essential nutrients from these low-yield compounds.

Objectives: An in-depth analysis of the African elephant gut microbiome could offer new insight into their unique digestive capabilities and assist in identifying a consortium of microorganisms capable of facilitating the digestion of fiber-rich compounds within a bioreactor.

Methods: Composition analysis of manure samples conducted by bomb calorimeter to access the viability of the samples as reactor feedstock. Bacterial and archaeal gut diversity was investigated using high-throughput Illumina sequencing of the 16S rRNA gene in samples collected from seven elephants.

Results: Energy release from elephant manure during combustion was calculated at 15.79 MJ/kg dry matter (DM), higher than cow manure (15.66 MJ/kg), a well-established substrate for biogas production. However, extremely high levels of both acid and neutral detergent fiber pose a challenge to complete degradation and energy release. Microbial composition analysis indicated a diverse prokaryotic community present in the elephant gut capable of high cellulose substrate digestion. Preliminary results suggest this community could aid in the treatment of fiber-rich compounds.

PT059 The Big Blue - Production of Specialised Metabolites by Marine Microalgae Grown Under 405 nm Light

Alison Hughes[1], Douglas McKenzie[2], Katherine Duncan[1]

[1]*University of Strathclyde, Strathclyde Institute of Pharmacy and Biomedical Sciences, Glasgow, United Kingdom*
[2]*Xanthella Ltd., Oban, United Kingdom*

Background: Microalgae are unsurpassed in terms of phylogenetic diversity and although their metabolites have been investigated for biofuels, they offer a relatively untapped source of specialised metabolites for nutraceuticals and biotechnological applications. Marine microalgae inhabit the euphotic ("sunlight") zone of the ocean as they require light for photosynthesis, which produces precursors used for their secondary (specialised) metabolism. Growing microalgal species at varying wavelengths of light can alter concentrations of these specialised metabolites produced, with astaxanthin – an industrially relevant pigment for aquaculture – production almost doubling when *Haematococcus pluvialis* was grown under a combination of blue and red LEDs. [1] It has also been demonstrated that 405nm light inhibits growth of airborne bacteria [2] without affecting growth of the microalgae *Chlorella sorokiniana* and could be used for open water or wastewater culturing systems.

Objectives: To evaluate the effect of 405 nm light on the growth, antibacterial activity, and metabolite production of phylogenetically diverse marine microalgae.

Methods: Microalgal strains grown in 1 L MicroPharos™ photobioreactors (Xanthella Ltd., UK) were extracted using ehtyl acetate and HP-20 resin. Crude metabolite extracts were screened against a panel of bacteria (*B. subtilis, E. coli, P. putida, P. aeruginosa*, *V. fischeri*) and comparative metabolomics was achieved using LC-MS/MS and molecular networking.

Results: Using the Global Natural Products Social (GNPS) molecular networking platform, a network comparing the induction and upregulation of metabolite production was compared across different wavelengths. The evaluation of antibacterial activity against common pathogens furthers the potential of microalgae as nutracueticals and feedstock for aquaculture.

PT060 Individual-based models to understand natural microbial communities

Rebeca Gonzalez-Cabaleiro[1], Valentina Gogulancea[2], Cindy Smith[1], Dana Ofiteru[2]

[1]*School of Engineering, Infrastructure and Environment, Glasgow, United Kingdom*
[2]*School of Engineering, Chemical Engineering, Newcastle upon Tyne, United Kingdom*

Background: Our ability to engineer processes in which we use microbial populations, is still limited: we lack knowledge on the mechanisms that control their structure and behaviour. Increasing our understanding will lead us to design more efficient biotechnological processes.

Objectives: To use our mathematical modelling capacity to describe the interactions between different microbial populations in natural communities.

Methods: We use a flexible and fully comprehensive Individual-based model to describe at the microscale level natural communities of microorganisms forming granules. In the model, each individual act as a micro-reactor. The model is flexible because allows the consideration of any metabolism and it is comprehensive because includes sub-models to describe in-detail the chemistry of the system (e.g. acid-basic reactions and liquid-gas transference).

Results: First, we have been able to describe the competition for oxygen of ammonia-oxidising bacteria and nitrite-oxidising bacteria in a granule. The model was able to predict the conditions to outcompete nitrite-oxidising bacteria. This is fundamental to optimize granule systems in which the aim is to remove the dissolved nitrogen growing annamox bacteria in the anaerobic core.

Second, the model has been used to describe anaerobic fermentative granules using glucose as substrate. The objective is to analyse the stratification that under specific environmental conditions has been observed in these granules. These simulations are still work on progress.

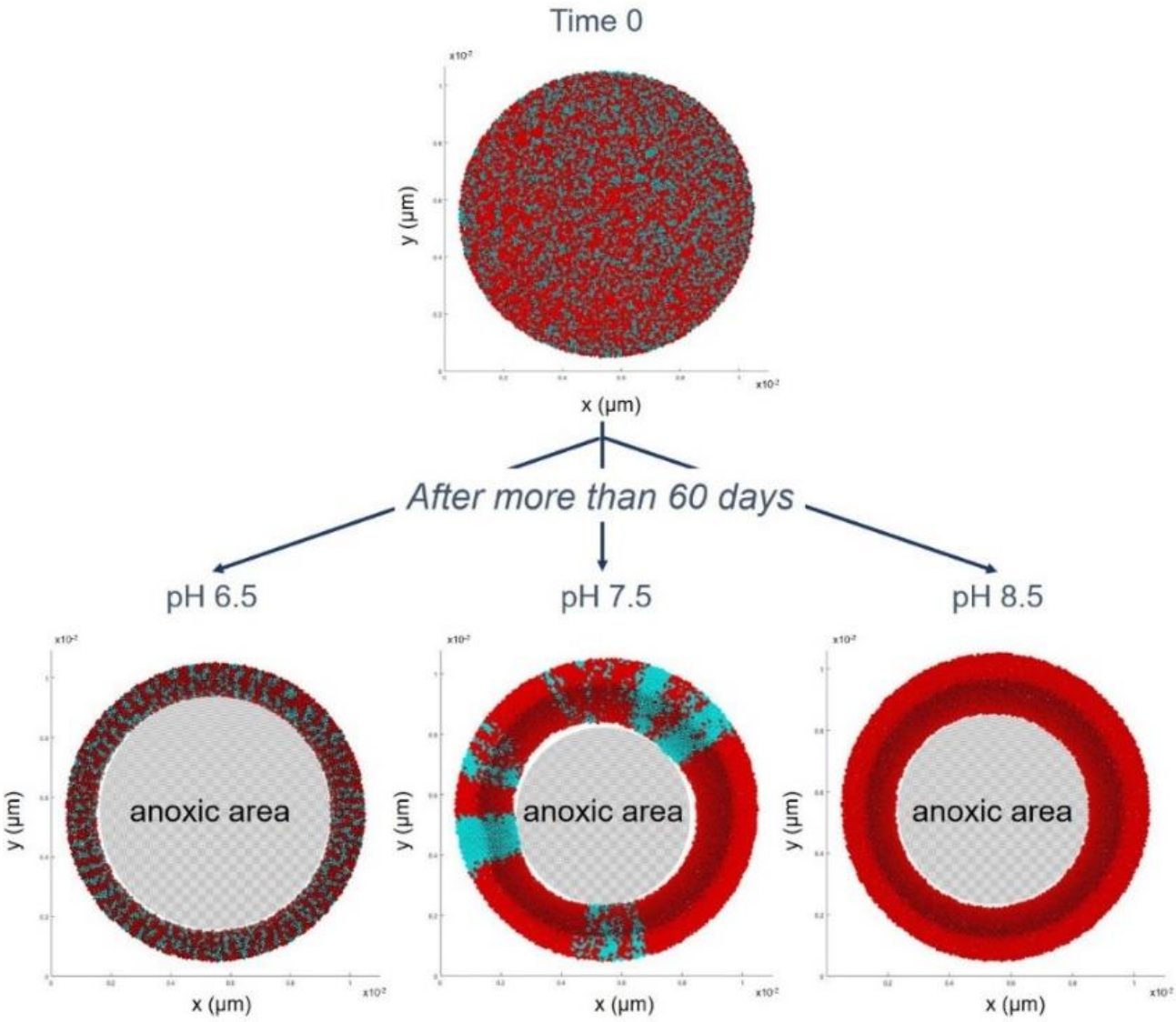


Figure 1 Simulation of nitrification in an aerobic granule: AOB in red and NOB in blue are competing for oxygen but collaborating in the nitrification process. At basic pH, AOB outcompete NOB bacteria.

PT061 Nucleic acid mimics and spectral imaging application to fluorescence in situ hybridization (FISH) for a high efficient multiplex detection of clinical pathogens

Ricardo Fernandes[1], Andreia Azevedo[1], Ana Rita Faria[2], Óscar Silvestre[2], Jana Nieder[2], Paula Sampaio[3], Nuno Azevedo[1], Carina Almeida[4]

[1]*Faculty of Engineering, University of Porto*
[2]*INL International Nanotechnology Laboratory, Braga, Portugal*
[3]*i3S Instituto de Investigação e Inovação em Saúde*
[4]*National Institute for Agrarian and Veterinary Research*

Background: The application of nucleic acid mimics (NAMs) have improved the performance of FISH methods for the detection of clinical pathogens. However, an important limitation of FISH techniques is the low number of distinguishable targets. The use of filters in fluorescence image acquisition limits the number of fluorophores that can be simultaneously differentiated. Recent advances in fluorescence spectral image acquisition have allowed the unambiguous identification of several fluorophores.

Objectives: In this work we aimed to combine NAM-FISH and spectral image analysis to develop and validate a new colour-coded FISH methodology that allows a multiplexed, robust and rapid detection of clinical pathogens.

Methods: We have selected eight fluorophores with distinct spectral properties and 8 clinical pathogens. For a better control of the thermodynamic parameters, locked nucleic acid/2'-O-methyl-RNA (LNA/2'OMe) probes were synthetized with the different fluorochromes. Universal Eubacteria probe sequences were used to rank the species/fluorochromes. After the hybridization, samples were analysed by confocal laser scanning microscopy coupled with a spectral imaging detector. A linear unmixing algorithm was applied to allow the differentiation of fluorophores. Final validation tests were performed using mixed populations to evaluate the technique potential for separating/quantifying the different targets.

Results: Strong variations on the signal intensity were found between species and between fluorochromes. However, this methodology allowed the establishment of balanced species/fluorochromes pairs and provided an unambiguous separation of eight fluorophores. Validation tests with different proportions of bacteria labelled with the different fluorophores have shown the method ability to correctly estimate differences in complex populations.

PT062 Hi-C Assigns Mobile Genetic Elements To Host Bacteria In Complex Microbial Communities

Iain Kean[1], Srishti Gupta[1], Lajos Kalmar[1], Andrew Grant[1], Mark Holmes[1]

[1]*Department of Veterinary Medicine, University of Cambridge, Cambridge, United Kingdom*

Background: Bacterial infectious diseases contribute to more than 54% of global infectious diseases and the ever-increasing magnitude of antimicrobial resistant (AMR) pathogens is a major concern for animal and public health worldwide. Acquired resistance traits are mostly contained on mobile genetic elements (MGEs). Next generation sequencing (NGS) has substantially increased our understanding of the microbiome, but fails to accurately identify the location of a particular gene of interest within any single host genome. Chromosome conformation capture and its derivative Hi-C are powerful techniques that allow the generation of 3D genome and transcriptome organisational maps.

Objectives: This study aims to demonstrate the ability to identify AMR genes within complex microbial communities, and localize MGEs to their host bacteria, without the need to culture, using NGS and bioinformatics.

Methods: *De novo* genomic assemblies were constructed from metagenomic and Hi-C NGS data from 100 pig faeces samples. After mapping Hi-C fragments to the contigs, pairwise inter-contig connections were used to define core-communities (CC) representing one prokaryotic genome or a group of very similar genomes.

Results: We identified 3075 AMR genes in 3590 CCs. No major differences were observed in taxonomic groupings compared to published data. Sequences assembled from our study clustered closely to published genomes. Comparing Organic and Conventional pig farms we observed higher numbers of AMR genes in pig faeces from conventional farms. The *lnuA* gene of *Lactobacillus johnsonii* and *Lactobacillus reuteri* appears to be on a plasmid and was observed only in samples from conventional farms.

PT063 The Syngulon PARAGEN collection: a standardized synthetic bacteriocin gene library for rapid in vitro and in vivo antimicrobial peptide production

Juan Borrero del Pino[1], Jason Bland[1], Mohamed El Bakkoury[1], Johann Mignolet[1], Philippe Gabant[1]

[1]*Syngulon*

Background: Bacteria possess a vast reservoir of bacteriocins, antimicrobial peptides whose potential as alternative to antibiotics, as biopreservatives or in other industrial applications has yet to be implemented. The increasing information generated by next generation sequencing allows for the identification of genes coding for bacteriocins in complex microbial communities. Recent advances in synthetic biology including cell-free synthesis or molecular cloning enable the production of a substantial number of such bacteriocins.

Objectives: In Syngulon we are developing a collection of more than 500 nucleotidic sequences of previously described and novel (PARAGEN 1.0) and/or fully synthetic (PARAGEN 2.0) bacteriocins. The aim is using these genes for the production of bacteriocins *in vitro* and *in vivo*, to generate a large set of active compounds ready to be tested against relevant pathogenic strains.

Methods: All genes in PARAGEN 1.0 and 2.0 code for the mature sequence of the bacteriocins. Such bacteriocin genes have been expressed under the control of the T7 promoter for the production of active bacteriocins by *E. coli* cell-free protein synthesis or by the industrial strain *Saccharomyces cerevisiae*.

Results: Over 65% of the bacteriocins synthesized using both approaches have showed antimicrobial activity, individually or in combination (“cocktails”) against different Gram + and Gram - strains of industrial and medical interest. This is part of our ongoing efforts in synthetic biology to use DNA information to produce bacteriocins, characterize their full potential, and find tailored solutions to meet the needs of our industrial partners.

PT064 Synthetic Phage-Inducible Chromosomal Islands, the Trojan Horse against AMR

Rodrigo Ibarra Chavez[1;2], Jose Penades[1], Jonathan M. Cooper[2]

[1]*University of Glasgow, Institute of Infection, Immunity and Inflammation, Glasgow, United Kingdom*
[2]*University of Glasgow, Division of Biomedical Engineering, Glasgow, United Kingdom*

Background: With the emerging global threat of antimicrobial resistance (AMR), diagnosis and treatment of infectious diseases become more challenging than ever. We need to consider new paradigms in therapy and innovative alternatives that can allow us to rapidly detect bacteria at the point-of-care. PICIs are ubiquitous and parasites of a wide range of hosts and prophages. The key innovation in our approach lies within the *modus vivendi* of the PICIs that hijack the phage machinery and exploit it to spread to other hosts.

Objectives: Here we use synthetic phage-inducible chromosomal islands (PICIs) to combine the two concepts of diagnostics and therapy to "seek & destroy" specific bacteria.

Methods: We use a wide range of molecular and microbiology methods to engineer our synthetic PICIs.

Results: We have engineered PICIs with synthetic gene circuits to enable tracking of pathogens. By assessing the viability and detection of cells, we show that PICIs can achieve greater transfer and sensitivity compared with phages, reaching a limit of detection of 20 CFU ml^{-1}. We have also adapted the detection protocol for their use in low-cost, portable paper devices. Further modifications prove that PICIs can be used as Trojan-horses to deliver lethal payloads into target cells, such as CRISPR-Cas9. These synthetic PICIs bring new advantages for the rational design of synthetic biology components. They can be used as a tuneable platform for the detection and elimination of the pathogen exploiting the pathogen to modify its genome specifically to produce a reporter (seek) and/or a killing switch (destroy).

PT065 Expression of Streptomyces davawensis rosB gene in riboflavin overproducing strain of the methylotrophic yeast Komagataella pastoris

Justyna Ruchala[1], Andriy Tsyrulnyk[2], Liubov Fayura[2], Hans Marx[3], Diethard Mattanovich[3], Dariya Fedorovych[2], Kostyantyn Dmytruk[2], Andriy Sibirny[1;2]

[1]*University of Rzeszow, Faculty of Biotechnology, Department of Microbiology and Biotechnology, Rzeszow, Poland*
[2]*Institute of Cell Biology, National Academy of Science of Ukraine, Lviv, Ukraine*
[3]*University of Natural Resources and Life Sciences, Vienna, Austria*

Background: Antibiotic roseoflavin and its biosynthetic precursor aminoriboflavin are produced by gram-positive bacteria *Streptomyces davawensis*. Aminoriboflavin shows a strong antibiotic effect on bacteria such as *Staphyloccus aureus*, *Bacillus subtilis* etc. being non-toxic to mammalian cells. Since aminoriboflavin is synthesized from flavin mononucleotide (FMN), which is formed from riboflavin, it is promising to apply riboflavin overproducers as basic strains for the construction of aminoriboflavin producers.

Objectives: The aim of this work was to achieve the heterologous expression of the key gene of aminoriboflavin biosynthesis rosB of S. davawensis, encoding 8-dimethyl-8-aminoryboflavin-5'-phosphate synthetase, in the engineered flavinogenic strains Komagataella pastoris.

Methods: Methods of microbial molecular genetics and biochemistry have been used.

Results: The chemically synthesized *rosB* gene with codons adapted to *K. pastoris* and native *FMN1* gene under control of strong promoters were introduced into the genome of *K. pastoris* riboflavin overproducing strain. Transformants of *K. pastoris* with the pr*TEF1-FMN1*-pr*GAP-rosB* expression cassette accumulated a high amount of a new yellow fluorescent compound in the culture medium. *K. pastoris* transformants expressing pr*GAP1-FMN1*-pr*TEF1-rosB,* pr*DAS2-FMN1*-pr*AOX1-rosB* and pr*GAP1-rosB* also produced this new product at lower extent. Spectra analysis of flavins accumulated in the culture medium of the pr*TEF1-FMN1*-pr*GAP-rosB* transformant showed two absorbance peaks near 445 and 478 nm corresponding to riboflavin and aminoriboflavin were detected. We suggest that the bacterial *rosB* gene was successfully expressed in the transformed *K. pastoris* strain leading to production of the roseoflavin biosynthetic precursor aminoriboflavin. To our knowledge, this is the first example of construction of the yeast strain producing bacterial antibiotic.

PT066 Mechanism of induced tolerance in E. Coli mediated by a toxin-antitoxin module

Irine Ronin[1], Nathalie Questembert-Balaban[1]

[1]*The Hebrew University in jerusalem, Racah Institute of Physics, Jerusalem, Israel*

Background: Dormant state of bacteria provides tolerance and survival under antibiotic treatment. During previous *in vitro* evolutionary experiments under intermittent antibiotic treatments, we repeatedly found mutations in the VapBC toxin-antitoxin (TA) module of *E.coli*. The mutations caused a prolonged lag time and increased antibiotic tolerance-by-lag (*tbl*).

Objectives: The objective of this research is to dissect the VapBC mechanism leading to tolerance**.**

Methods: We built tightly regulated synthetic systems to analyze the mechanism of growth inhibition in the mutants. By measuring quantitative parameters of the VapBC module in batch cultures and at the single cell level, we were able to account for the effect of the mutations on the growth arrest and tolerance.

Results: Our results shed new light on the link between TA modules and tolerance-by-lag.

PT067 The role of fibronectin nanopattern in limiting staphylococcus aureus adhesion to biomaterial surfaces

Heba Khateb[1], Rikke Meyer[1], Duncan Sutherland[1]

[1]*Aarhus University, iNANO, Aarhus, Denmark*

Background: Bacterial adhesion to materials surfaces is of critical importance in many application areas, in particular in controlling infection related to medical implants. A range of bacteria can adhere specifically to proteins from the extracellular matrix found in tissue enabling infection and biofilm formation.

Objectives: Here we study specific interactions of ***staphylococcus aureus*** with fibronectin (Fn) adhered to materials surfaces

and address the following questions:

-**Does the nanoscale distribution of the Fn influence the adhesion?**
-How does Fn adherent layers influence bacterial adhesion to nanoscale topography?

Methods: We utilize colloidal lithography to prepare patterns of Fn (from 0.1 µm - 1 µm) in

a protein rejecting background of silicon dioxide coated with a precoated layer of Fn and study bacterial adhesion under flow.

Results: The results indicate that the adhesion of S. Aureus to surface adsorbed Fn is altered at sub micrometer patterns in a size dependent manner with a threshold for between 300nm patches. Above the threshold, increased adhesion was observed for larger patterns. Moreover, there was not a significant difference between the 300 and 500 nm in number of adherent bacterial cells. However,, interestingly, 800 nm patches were more influenced for a bacterial adherence. Meanwhile, no significant difference has been noticed between the former size and 1000nm on the adherence number.

former size and 1000nm on the adherence number.

PT068 Evaluation of transcriptional factors involved in the regulation of glucose and xylose metabolism and fermentation in yeast Ogataea polymorpha

Olena Kurylenko[1], Roksolana Vasylyshyn[1], Justyna Ruchala[2], Kostyantyn Dmytruk[1], Andriy Sibirny[1;2]

[1]*Institute of Cell Biology, NAS of Ukraine, Department of Molecular Genetics and Biotechnology, Lviv, Ukraine*
[2]*University of Rzeszow, Department of Microbiology and Biotechnology, Rzeszow, Poland*

Background: Glucose and xylose are the most abundant sugars of lignocellulose which represents an important renewable feedstock that can be converted to ethanol. One of the bottlenecks in lignocellulose conversion to ethanol is inefficient fermentation of xylose as compared to glucose by the majority of the known microorganisms. *Ogataea (Hansenula) polymorpha* is one of the most thermotolerant xylose-fermenting yeast species reported to date. Several metabolic engineering approaches have been successfully demonstrated to improve high-temperature alcoholic fermentation by *O. polymorpha*. Further improvement of ethanol production from xylose in *O. polymorpha* depends on the identification of bottlenecks in xylose conversion pathway to ethanol.

Objectives: Recently, the Cat8 was described as the first transcriptional factor involved in regulation of xylose alcoholic fermentation in *O. polymorpha*. Currently, our study is focused on identification of other genes coding for putative transcriptional factors in *O. polymorpha*.

Methods: Methods of molecular genetics and biochemistry.

Results: Effect of deletion of the genes coding for transcriptional factors *MIG1, MIG2, TUP1, HAP4A, HAP4B* on metabolism and fermentation of glucose and xylose was studied. Moreover, recombinant strains overexpressing *HAP4A*, *HAP4B*, *ZNF1*, *ASG1*, *SEF1*, *TUP1* genes under control of strong constitutive promoter or native promoters in the frame of the multicopy plasmid were constructed. Several constructed knock out mutants were characterized by activation of xylose alcoholic fermentation. The *tup1Δ* mutant was characterized by substantial increase of ethanol production during xylose alcoholic fermentation, however, practically was unable to ferment glucose. Moreover, overexpression of the *TUP1* gene resulted in decreased ethanol production from xylose.

PT069 Comparing traditional techniques with molecular methods in diagnosing Prototheca wickerhamii: a case report and review

Shin-Yi Tsai[1;2;3], Yu-Ting Tina Wang[1], Chon-Fu Lio[1], Kam- Hang Leong[1], Chien- Feng Kuo[4]

[1]*Mackay Memorial Hospital, Department of Laboratory Medicine, Taipei City, Taiwan*
[2]*Mackay Medical College, Department of Medicine, New Taipei city, Taiwan*
[3]*Johns Hopkins University, Baltimore, United States*
[4]*Mackay Memorial Hospital, Institute of Infectious Diseases, Taipei City, Taiwan*

Background: *Prototheca Wickerhamii* infection is a rare, opportunistic disease caused by algae belonging to genus *Prototheca*. Human protothecosis is mostly associated with immunocompromised patients and presents in at least three main clinical forms, localized cutaneous disease, olecranon bursitis and disseminated disease.

Objectives: To avoid a delayed or missed diagnosis, laboratorians and clinicians must be aware of the unspecific clinical manifestations and microscopic features of *Prototheca* spp..

Methods: A 73-year-old man with multiple underlying conditions presented to the Emergency department with erythema of bilateral forearms for more than 4 weeks. The cutaneous symptoms were predominantly vesiculobullous and ulcerative lesions with purulent exudates. The patient had applied traditional herbal medications.

Blood agar plate (BAP), chocolate agar (CHOC), Sabouraud's dextrose agar (SDA), wet mount culture, Vitek 2, matrix-assisted laser desorption ionization-time of flight (MALDI-TOF), and histopathological examinations were performed. The molecular diagnosis required sequencing in both forward and reverse directions of the D1/D2 variable domain of the 28S rDNA region, which was amplified with primers.

Results: The CHOC and SDA cultures showed smooth tiny colonies. Large Gram-positive spherical cells that varied in size and resembled yeast were observed. (Fig.1) The BAP examination yielded cream-colored and yeast-like colonies. The histopathological examination showed periodic acid–Schiff positive round organisms ranging from 8- 30 um in diameter. Sporangia with endospores in a morula-like pattern were observed by using lactophenol cotton blue stain and a wet mount. (Fig.2) The Vitek 2 and MALDI-TOF examinations indicated strong results *for P. wickerhamii*, which was proven by sequencing.

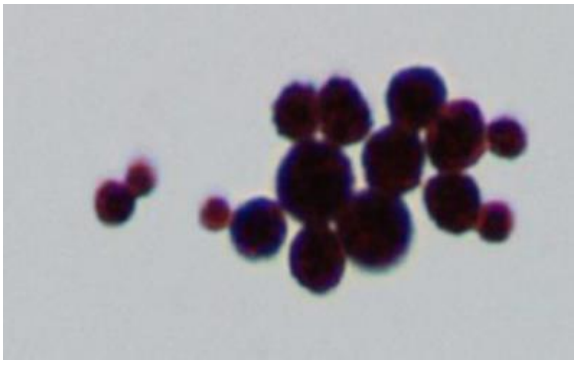


Fig.1

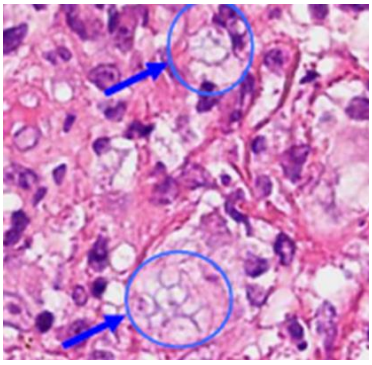


Fig.2

PT070 Real-time assessment of metabolic activity of microbes and microbial biofilms using fluorescent probes

Subhanjan Mondal[1], Said Goueli[1], Matt Styles[1], Nathan Feirer[1], Hui Wang[1], James Cali[1], Poncho Meisenheimer[1], Wenhui Zhou[1]

[1]Promega Corporation, R&D, Madison, United States

Background: Continuous non-lytic assessment of microbial metabolic activity using fluorescent probes offers distinct advantages over endpoint assays. Available technical options suffer insufficiencies, like non-selectivity of esterase substrate fluorescein diacetate (FDA) or cytotoxicity of tetrazolium dye (CTC).

Objectives: We hypothesized that attachment of a self-cleavable fluorescent quencher to a cell permeable non-toxic fluorescent dye would overcome these problems. Spontaneous intracellular cleavage that is dependent on cellular respiration would lead to release of the processed fluorescent dye in the medium.

Methods: The probe was synthesized and tested with various abiotic factors and found it to be non-fluorescent under all conditions tested, in contrast FDA fluoresced in the presence of alkaline pH, various microbial culture medium, positively charged amino acids and dead cell lysates. We tested microbial metabolic activity using the probe under various minimal and rich media and we observed differences in metabolic activity under different growth conditions. It is noteworthy that we observed considerable metabolic activity in isotonic solution, phosphate buffer and various minimal media which did not support rapid growth indicating that culture-independent viability assessment of microbes is possible using this probe. Next, we tested the ability of the dye to enter within *Pseudomonas* and *E. coli* biofilm and measure cellular metabolic activity. The probe was able to enter biofilm from both strains and reported metabolic activity as well as effectiveness of chlorohexidine and glutaraldehyde in controlling biofilm.

Results: The fluorescent probe offers real-time assessment of microbial metabolic activity and lays the foundation for development of novel microbial assay methods.

PT071 *Flavobacterium IR1* as a model organism for structural colour

Laura Catón Alcubierre[1], Villads Egede Johansen[2], Colin J. Ingham[3], Silvia Vignolini [1]

[1]*University of Cambridge, Department of Chemistry, Cambridge, United Kingdom*
[2]*Cambridge University, Department of Chemistry, Cambridge, United Kingdom*
[3]*Hoekmine B.V., Utrecht, Netherlands*

Background: Structural colour (SC) is a consequence of light interacting with ordered nanostructures to reflect intense, angle-dependent hues. Despite the frequency and diversity of SC in living organisms, there is little knowledge of the underlying genes. Within the Bacteroides-Flavobacterium-Cytophaga SC is common in the coastal environment and the soil [1].

Objectives: Optical properties and phenotype of wild-type and mutated bacterial colonies to understand how genes regulate the colour of *Flavobacterium* strain Iridescent 1 (IR1) colony.

Methods: Transposon mutagenesis was used to identify genes that modulate SC in IR1, colony organization and photonic response were studied by scanning electron microscopy and optical goniometer.

Results: IR1 rapidly self-assembles into a 2D photonic crystal on hydrated surfaces to create complex and beautiful patterning. Consequently, IR1 colonies are able to display intense, angle-dependent colours when illuminated with white light. Genes involved in gliding motility, the stringent response, polymer interactions and genes with no previously known role were found to be important [2]. This work sets the stage for a more widespread genomics effort to understand the evolution of both SC and structures in nature.

[1] Kientz, B., Agogué, H., Lavergne, C., Marié, P., & Rosenfeld, E. (2013). Isolation and distribution of iridescent Cellulophaga and other iridescent marine bacteria from the Charente-Maritime coast, French Atlantic. *Systematic and applied microbiology*, *36*(4), 244-251.

[2] Johansen VE/Catón L, Hamidjaja R, Oosterink E, Wilts BD, Rasmussen TS, Sherlock MM, Ingham CJ and Vignolini S (2018). Living colours: Genetic manipulation of structural colour in bacterial colonies. *PNAS* , *115*(11) 2652-2657.

PT072 Riboflavin excretase is important for riboflavin overproduction in the flavinogenic yeast Candida famata

Andriy Sibirny[1;2], Andriy Tsyrulnyk[1], Dariya Fedorovych[1], Justyna Ruchala[2], Kostyantyn Dmytruk[1]

[1]*Institute of Cell Biology, NAS of Ukraine, Lviv 79005 Ukraine, Molecular Genetics and Biotechnology, lviv, Ukraine*
[2]*University of Rzeszow, Microbiology and Biotechnology, Rzeszow, Poland*

Background: Microorganisms possess transport systems for uptake and excretion of metabolites. The role of excreting transporters in control of metabolite synthesis is poorly understood. Flavinogenic yeast *Meyerozyma guilliermondii* was found to possess riboflavin excretase. Other flavinogenic yeasts, like *Candida famata*, presumably also contain riboflavin excretase as all overproduced riboflavin efficiently is extruded from the cells and is accumulated in cultural medium.

Objectives: To express gene for riboflavin excretase in the riboflavin overproducing strains of *C. famata* and studying flavinogenic properties of the resulted transformants

Methods: Methods of molecular genetics and biochemistry.

Results: Flavinogenic yeasts contain homologs of the mammal *BCRP* gene coding the protein responsible for secretion of riboflavin to the milk. *BCRP* gene homolog was isolated from genome of *Debaryomyces hansenii* and expressed under control of own strong constitutive *TEF1* promoter in the riboflavin overproducing strain of the yeast *C. famata*. Resulted transformants were stable and exhibited overexpression of *D. hansenii BCRP* gene homolog and produced 1.3 – 1.5 times more riboflavin relative to the parental overproducing strain. The cloning of *BCRP* gene homolog from *C. famata* is under way. The role of riboflavin excretase in riboflavin oversynthesis will be discussed.

PT073 Fungicidal and fungistatic activity of Minthostachys mollis (muña) against Candida albicans

Mauricio Armas-Gálvez[1], Luis Neyra-Espinoza[1], Miguel Angel Aguilar-Luis[2;3], Hugo Carrillo-Ng[3], Ronald Aquino Ortega[2;3], Juana del Valle-Mendoza[2;3]

[1]Universidad Peruana de Ciencias Aplicadas, School of Dentristry, Faculty of Health Sciences, Lima, Peru
[2]Universidad Peruana de Ciencias Aplicadas, School of Medicine, Research Center and Innovation of the Faculty of Health Sciences, Lima, Peru
[3]Instituto de Investigacion Nutricional, Molecular Biology and Biochemistry Laboratory, Lima, Peru

Background: Oral candidiasis is one of the most prevalent micotic infections that affects the oral cavity. Currently, *Candida albicans* has developed resistance to multiple antimicotics available. The use of medicinal plants has emerged as an alternative therapy to the use of synthethic antimicrobials. *Minthostachys mollis* (muña), is a native Peruvian plant that grows in the Andes. Previous studies, have demonstrated its antibacterial effects against pathogens such as *Helicobacter pylori, Salmonella typhi and Pseudomonas aeruginosa*. However, its effect against oral pathogens has not been studied yet.

Objectives: To evaluate the fungistatic and fungicidal effect of the methanol extract of *Minthostachys mollis* (muña) against *Candida albicans* ATCC 10231.

Methods: Three separate *Minthostachys mollis* methanol extracts were prepared with the leaves, stems and roots (1:2, w/v). The fungistatic effect was evaluated using the cup-plate agar diffusion method, using clotrimazole as a postive control. The minimum inhibitory concentrarion (MIC) was determined using the microdilution method as described by the CLSI. The cytotoxicity assay ($CC_{50\%}$) was determined using the MTT assay with Madin-Darby canine cells.

Results: The leaves, stems and roots methanol extract showed fungistatic effect against *Candida albicans*, with a inhibitiory halo of (47.72 ± 6.67) mm, (46.58 ± 6.42) mm and (26.18 ± 7.03) mm respectively. While, clotrimazole showed an inhibitory halo of 52mm. The MIC of the leaves, stems and roots were, 46.87mg/ml, 93.75mg/ml and 1500mg/ml, respectively. The $CC_{50\%}$ were 150 µg/ml, 200 µg/ml and 300 µg/ml, respectively.

PT074 Green bioproduction of monoterpenoid (E)-geranic acid from geraniol by genetically engineered Acinetobacter sp. Tol 5 in gas phase

Atsushi Usami[1], Masahito Ishikawa[1], Katsutoshi Hori[1]

[1]*Nagoya University, Engineering, Japan*

Background: Gas phase reaction using solid/gas biocatalysis allows for the creation of environmentally friendly industrial processes [1]. However, previous studies have not reported the production of valuable monoterpenoid. *Acinetobacter* sp. Tol 5, a Gram-negative bacterium, is readily immobilized onto various support materials due to its high, non-specific adhesiveness and can be used as the biocatalysist in gas phase reaction [2]. Furthermore, Tol 5 transformant expressing geraniol dehydrogenase (*geoA*), Tol 5 (pGeoA), produces (*E*)-geranic acid (GA), an industrially important monoterpenoid, from geraniol by a typical liquid phase reaction [3].

Objectives: This study aimed to produce GA from geraniol by gas phase reaction using a genetically engineered Tol 5 cells.

Methods: To increase the intracellular accumulation of GA, *fadD4*, a putative gene involved in GA degradation, was eliminated from Tol 5's chromosome. Tol 5 (pGeoA) and Δ*fadD4* mutant harboring *geoA*, Δ*fadD4* (pGeoA), were immobilized onto a polyurethane-foam, transferred into a vial supplemented with geraniol, and incubated at 28°C for 6 days. GA production was measured by Headspace-Gas Chromatography-Mass Spectroscopy.

Results: GA production was observed in both gas phase reactions using Tol 5 (pGeoA) and Δ*fadD4* (pGeoA) cells. Expectedly, Δ*fadD4* (pGeoA) cells accumulated more GA than Tol 5 (pGeoA) cells. Our data indicates the feasibility of GA production by gas phase reaction using genetically engineered Tol 5 cells.

References

1. Kulishova LM, et al. (2017) *Biochem*. 82, 95-105.

2. Yoshimoto S, et al. (2017) *Microb. Cell Fact.* 16, 123-133.

3. Usami A, et al. (2018) *Biosci. Biotechnol. Biochem.* 82, 2012-2020.

PT075 Ribonuclease III deletion and dsRNA synthesis in the model bacterium - Sinorhizobium meliloti

Simon Goodfellow[1;2], Daai Zhang[1;2], Ming-Bo Wang[2], Ren Zhang[1]

[1]*University Of Wollongong, Biological Sciences, Gwynneville, Australia*
[2]*CSIRO, Agriculture and Food, Black Mountain, Australia*

Background: A limitation in the deployment of RNA interference (RNAi)-based therapeutics has been a lack of means to efficiently synthesize and apply double-stranded RNA (dsRNA). One method of overcoming this involves the use of bacteria to synthesize RNAi-effectors in culture, which can then be purified and applied. This requires the deletion of double-stranded RNAses, namely – Ribonuclease III (RNAse III). An existing *E. coli* strain has been shown to express dsRNA at high levels and hence is widely used.

Objectives: This study sought to investigate if deletion of RNase III genes in other bacteria would also allow for efficient dsRNA production. We started by testing *Sinorhizobium meliloti* – a bacterial species widely used in molecular biology.

Methods: The *S. meliloti* RNAse III gene – *rnc*S was deleted using recombineering-mediated fusion of a selectable marker with the rncS ORF. The wild type and resulting mutant were transformed with plasmids driving synthesis of a hairpin structured dsRNA (hpGUS) targeting a β-glucuronidase (GUS) reporter gene. Yield and integrity of hpGUS was assessed by northern blot and compared with their counterpart *E. coli* strains.

Results: hpGUS RNA concentration was higher in *rnc*- strains than wild-type strains. However, hpRNA load in all *S. meliloti* strains was lower than in the existing RNase III-deficient *E. coli* strain. The differences have been putatively attributed to a combination of differences in plasmid copy number, presence/absence of a T7 RNA polymerase expression system, and endogenous RNAse activity. Modifications are underway to optimize the level of hpRNA or dsRNA accumulation in the bacterium.

PT076 Improvement of rational design of ectoine-producing strains by development of a new generation metabolic model (ME-Model) of the extremophile Chromohalobacter salexigens

Francine Piubeli[1], Joaquin J Nieto[1], Lourdes Martinez-Martinez[1], emilia Naranjo[1], Rosa García-Valero[1], Manuel Salvador[2], Carmen Vargas[1], Montserrat Argandoña[1]

[1]*Faculty of Pharmacy, Department of Microbiology and Parasitology, Sevilla, Spain*
[2]*Faculty of Health and Medical Sciences, Guildford, United Kingdom*

Background: *Chromohalobacter salexigens* is a halophilic bacterium that synthesizes ectoines, bio-stabilizing compounds of great biotechnological interest, in response to increasing salinity and temperature. To facilitate the rational design of improved strains of this bacterium for ectoine overproduction, a genomic scale metabolic model (M-model) was developed (*i*FP764). However, new generation models to enhance the predictive capacity of M-models are desirable, such as the transcription and translation model (E-Model), which takes into account the energy expenditure necessary for the synthesis of macromolecules (RNA and proteins) related with metabolic enzymes through the macromolecular machinery. This model could be further coupled to a previous M-Model yielding an ME-Model, with better-quality prediction capacity.

Objectives: To develop an E-Model of *C. salexigens* to be later connected to the metabolic Model *i*FP764 (M-Model), generating an improved ME-Model.

Methods: The transcriptional and translational network of *C. salexigens* was reconstructed by an exhaustive genomic analysis. Genes involved in the synthesis of RNA and proteins were identified and associated to different subsystems. Transcriptional units (TUs) containing the selected genes as well as the sigma factor/s that control each TU, were defined by *in silico* analysis of operons and promoters. This information served to construct custom Python modules that joined with reaction-templates, allowed a semi-automatic formulation of stoichiometric reactions for each transcription and translation phases and subsequently, obtaining a high complexity mathematical matrix (E-Model).

Results: An E-Model of *C. salexigens* with more than 5,000 components and 4,695 reactions belonging to 18 different subsystems has been achieved.

PT077 Ensuring sustained genetic stability and productivity during continuous culture of E. coli for the bio-production of citramalate

Charlotte Green[1], Andrew Yiakoumetti[1], Gill Stephens[1], Alex Conradie[1]

[1]*University of Nottingham, Chemical and Environmental Engineering, Nottingham, United Kingdom*

Background: Although fed-batch fermentations are cost-effective for bio-production of high value chemicals, limitations of reactor size and process downtime make them less attractive for manufacturing commodity chemicals. Continuous fermentation provides a potential solution, however poor genetic stability of metabolically engineered production strains limits process longevity. The specific problem is that non-productive mutants appear and outcompete productive, engineered cells, due to their more efficient conversion of substrate to biomass.

Objectives: We developed and tested genetically stable *E. coli* strains which express *cimA3.7*, enabling the production of citramalate, a non-toxic precursor to methacrylic acid.

Methods: We screened the Anderson promoter series to obtain optimised constitutive expression of *cimA3.7* from a plasmid, allowing high productivity, without the requirement of an inducer. We then tested plasmid stability in lab-scale continuous culture, and finally made and tested strain improvements to increase genetic stability.

Results: The plasmid was lost after 10 generations under glucose-limitation, and 30-40 generations under phosphate-limitation, resulting in loss of citramalate production. To ensure plasmid maintenance, we incorporated the essential gene, *infA*, into the expression plasmid and deleted *infA* from the chromosome. This strain retained the plasmid, but large deletions of plasmid DNA occurred instead, resulting in loss of citramalate production over a similar timescale. By contrast, a single chromosomal integration of *cimA3.7* resulted in stable citramalate production for over 150 generations, but with lower titres than the plasmid system. We are now extending our strategies to prevent plasmid structural instabilities, and to increase the productivity of the chromosomally integrated *cimA3.7* cassette system.

PT078 Metabolic engineering of Escherichia coli to produce astaxanthin

Seon Young Park[1;2], Robert Binkley[1], Won Jun Kim[1;2], Mun Hee Lee[1;2], Sang Yup Lee[1;2;3]

[1]Korea Institute of Advanced Science and Technology, Chemical and Biomolecular Engineering, Daejeon, Korea, Rep. of South
[2]Korea Institute of Advanced Science and Technology, Systems Metabolic Engineering and Systems Healthcare Cross-Generation Collaborative Laboratory, Daejeon, Korea, Rep. of South
[3]Korea Institute of Advanced Science and Technology, BioProcess Engineering Research Center and BioInformatics Research Center, Daejeon,

Background: Astaxanthin is a reddish keto-carotenoid which is found in many microbes and marine organisms. Because of its color and strong antioxidant property, it has been utilized in various industries

Objectives: Compared to the chemically synthesized astaxanthin, biologically produced astaxanthin is more stereoselective and more suitable for human consumption. In this study, *Escherichia coli* was chosen as a chassis strain to efficiently produce astaxanthin.

Methods: First, the expression levels of β-carotene biosynthetic genes were optimized. After that, various fusion tags were attached to the truncated BKT from *Chlamydomonas reinhardtii* (trCrBKT) to improve its stability and membrane binding ability. For increasing astaxanthin producing flux, amplification gene targets were identified by *in silico* flux variability analysis. Additionally, *hok/sok* system was introduced to stabilize the expression plasmid during fed-batch fermentation.

Results: After examining eight different fusion tags, signal peptide of *E. coli* OmpA at N-terminus and *E. coli* TrxA at C-terminus were selected and simultaneously fused to trCrBKT which resulted in 2.08-fold increased in astaxanthin titer. After optimization of culture conditions and screening the amplification gene targets, the engineered strain produced 432.82 mg/L of astaxanthin by fed-batch fermentation. The introduction of *hok/sok* system rather decreased the titer to 385.04 mg/L, however, the requirement of high concentration of antibiotics to maintain the expression plasmid was removed. [This work was supported by the Technology Development Program to Solve Climate Changes on Systems Metabolic Engineering for Biorefineries (Grants NRF-2012M1A2A2026556 and NRF-2012M1A2A2026557) from the Ministry of Science and ICT through the National Research Foundation (NRF) of Korea].

PT079 Development of non-virulent non-resistant bacterial chassis derived from strain PAO1 of Pseudomonas aeruginosa

Paulo Juarez[1], Melanie Grosjean[1;2;3], Sophie Guénard[1], caroline giraud [4], Patrick Plésiat[2;3], Cédric Muller[1]

[1]*Smaltis, R&D Department, Besançon, France*
[2]*UMR 6249 ChronoEnvironnement, Bacteriology, Besançon, France*
[3]*Centre National de Référence, Résistance aux Antibiotiques, Besançon, France*
[4]*Université de Caen - Normandie, U2RM: Unité de Recherche Risques Microbiens, Caen, France*

Background: Development of genetically-modified bacterial chassis is nowadays of interest to study complex cellular responses. In this context, targeting non-essential genes can be useful to achieve such objective. We worked with *Pseudomonas aeruginosa* which is considered as a model organism to study antibiotic resistance and bacterial pathogenesis.

Objectives: bacterial chassis derived from WT-strain PAO1 were developed by genomic editing to study mechanisms such as regulation of RND efflux pumps, novel mechanisms of antibiotic resistance or virulence. Moreover, strain GB01 is described to be used as a bacterial host in several molecular biology experiments.

Methods: Gene deletions were performed by homologous recombination. Bacterial growth was evaluated in different conditions. Standard protocols were used for drug-susceptibility testing and virulence factors. Cytotoxicity and adhesion were measured on murine macrophages J774A.1 and human pulmonary cells A549 respectively. Finally, *in vivo* tests using *Galleria mellonella* larvae were performed to evaluate the pathogenicity of strains.

Results: Genes related to efflux pumps, intrinsic mechanisms of resistance and virulence factors were targeted to create strains SM-Eff, SM-Res, and SM-Vir respectively. Moreover, strain GB01, deleted from all these genes and from genes related to cytotoxicity and adhesion, was also developed. All strains showed similar bacterial growth. Strains SM-Res, SM-Eff and GB01 presented increased susceptibility to antibiotics currently used as plasmid cassettes. *In vitro* tests of virulence, cytotoxicity and adhesion showed that only SM-Vir and GB01 presented a significant reduction of these traits. Finally, *in vivo* tests showed that strain GB01 killed 52% less larvae in comparison to strain PAO1.

PT080 The response of soybean plants to the co-inoculation with Delftia sp. JD2 and Bradyrhizobium elkanii U1301 and U1302

María Morel[1], Braulio Riviezzi[1], Agustina Pereira[1], Célica Cagide[1], Cristina Olivaro[2], Susana Castro Sowinski[1;3]

[1]Biological Research Institute Clemente Estable, BIOGEM, Montevideo, Uruguay
[2]Sede Tacuarembó Universidad de la República, Espacio de Ciencia y Tecnología Química / , Tacuarembo, Uruguay
[3]Faculty of Sciences UdelaR, Sección Bioquimica, Montevideo, Uruguay

Background: Delftia sp. JD2 is a plant-growth-promoting bacterium that produces 3-indol-acetic acid and increases plant growth, nodulation rate and grain yield of Glycine max (soybean) when is co-inoculated with bradyrhizobial strains.

Objectives: The aims were to identify the profiles of specialized metabolites from roots exudates of soybean co-inoculated with bradyrhizobia and JD2, and analyze their role in the interaction.

Methods: A specific design of the hydroponic system was developed with glass bottles and filter paper. Creases were made in the paper to let the root growth through the liquid while the seeds remain in the top during the seven days-invitro experiments. Radicules of pre-germinated seeds were inoculated/co-inoculated with Bradyrhizobium elkanii U1301+U1302 and Defltia sp. JD2. A series of filtrations allowed to recover the low molecular weight compounds (LMWC) and the macromolecules, separately. Organic acids from LMWC fraction were primarily analyzed by GC-MS and later by LC-MS, while proteins were extracted from the macromolecules fraction by ultra-diafiltration and analyzed by a ¨bottom up¨ proteomic approach.

Results: The primary phase of the project was completed with the design of a hydroponic experiment that allows the plant growth in sterile conditions and the recovering of the metabolites exudates by plants and bacteria into the liquid media. We detected a higher secretion of LMWC in exudates during co-inoculation. The changes in the profiles of LMWC, proteins, and flavonoids involved in an efficient rhizobial infective process, such as genistein, daidzein, and coumestrol, during the first days of the bacteria-plant interaction will be discussed.

PT081 Marine Dioxygenases: Microbes make a meal of oil

Julieta Rodríguez[1], Katya Ornelas Ocampo[1], Karla Sofia Millán López[1], José Luis Rodríguez Mejía[1], Luis Felipe Muriel Millán[1], Liliana Pardo López[1]

[1]*Instituto de Biotecnología Unam, Cuernavaca, Mexico*

Background: Catechol dioxygenases play a central role in the degradation of aromatic compounds, catalysing the chemically difficult aromatic ring-cleavage reaction. Catechol oxidation can occur through the ortho and meta pathways, catalysed by C12DO (catechol 1,2-dioxygenase) and C23DO (catechol 2,3-dioxygenase), respectively. Microorganisms that contain catechol dioxygenases are able to utilize aromatic molecules, as their sole source of carbon and energy, not only to survive in polluted areas, but in doing so, to decontaminate the soils.

Objectives: The cloning, expression and characterization of two catechol dioxygenases from *Pseudomonas* strains obtained from water samples of the Gulf of Mexico for its use in bioremediation.

Methods: The activity of both catechol dioxygenases was assayed spectrophotometrically, by monitoring changes in the product formation at 260 and 375 nm when catechol was used as a substrate. C12DO and C23DO genes from *Pseudomonas* samples were amplified and cloned into an expression vector. The recombinant proteins were purified and the biochemical characterization of the enzymes is being tested

Results: We analyzed the activities of C12DO and C23DO in 40 bacterial isolates and 160 bacterial consortia that were able of utilize crude oil and kerosene as the only carbon source. Two *Pseudomonas* sp. were isolated, we identified that the ortho or meta pathway was induced when benzoic acid or phenol was present in the culture media. We are currently working on the enzymatic assays at different conditions of temperature, pH and salinity to determine the optimal ranges of these dioxygenases and carrying out enzyme immobilization test using alginate beads.

PT082 A novel alkaline protease with potential application for eco-friendly enzymatic dehairing in the leather industry.

Cheng Zhou[1], Yanfen Xue[1], Yanhe Ma[1]

[1]*Institute of Microbiology, Chinese Academy of Sciences, State Key Laboratory of Microbial Resources, Beijing, China*

Background: Alkaline proteases have potential applications in the leather industry. However, there are still significant limitations in exploiting these enzymes for industrial used.

Objectives: Discover novel alkaline proteases with high activity and complementary properties suitable for dehairing processing in leather industry.

Methods: Gene cloning, secretory expression, characterization and dehairing evaluation.

Results: A novel alkaline protease (AprA) gene from alkaliphilic *Idiomarina* sp. C9-1 was cloned and expressed. The purified AprA and its pre-peptidase C-terminal (PPC) domain-truncated enzyme (AprA-PPC) showed maximum activity at pH 10.5 and 60°C, and were active and stable in a wide range of pH and temperature. Ca^{2+} significantly improved the thermostability and increased the optimal temperature to 70°C. Both AprA and AprA-PPC showed good tolerance to surfactants and oxidizing and reducing agents. We found that the PPC domain contributed to AprA activity, thermostability and surfactant tolerance. With casein as substrate, AprA and AprA-PPC showed the highest specific activity of 42567.1 U mg^{-1} and 99511.9 U mg^{-1}, respectively. Secreted expression of AprA-PPC in Bacillus subtilis after 48 h cultivation resulted in yield of 4935.5 U ml^{-1} with productivity of 102.8 U ml^{-1} h^{-1}, which is the highest reported in literature to date. Without adding any lime or sodium sulfide, AprA-PPC was effective in dehairing cattle hide and skins of goat, pig and rabbit in 8–12 h without causing significant damage to hairs and grain surface. Our results suggest that AprA-PPC may have great potentials for ecofriendly dehairing of animal skins in leather industry.

PT083 Bioinformatics analyses of IDL regions of eubacterial SSB proteins

Tina Paradžik[1], Želimira Filić[1], Dušica Vujaklija[1]

[1]*Ruđer Bošković Institute, Department for physical chemistry, Zagreb, Croatia*

Background: SSB (**s**ingle-**s**tranded DNA **b**inding) proteins are indispensable for survival in all forms of life. These proteins bind and protect single-stranded DNA (ssDNA) during DNA recombination, replication and repair. The eubacterial SSB proteins act as a homotetramers. Each monomer has two distinct domains: N-terminal domain with a conserved oligonucleotide-oligosaccharide fold (OB-fold) responsible for ssDNA binding, and C-terminal domain (Ct) with conserved tip. Ct is intrinsically disordered (IDL) and play important role in mediating SSB-protein interactions. Repetitive motifs, PXXP and GGX, which contibutes to elastomeric properties were found within IDL regions of SSB proteins.

Objectives: To analyse the conservation of PXXP and GGX elements in IDL regions with respect to GC content of bacterial SSBs. To examine the impact of increased glycine/proline ratio on the variations of these motifs in actinobacterial SSBs.

Methods: SSB sequences were collected from genomes covering a wide range of GC content from 14 bacterial phyla. The ML trees were constructed for SSB sequences and corresponding 16S rRNA genes. The amino acids (aa) content of OB folds and IDL regions were subsequently analysed. Variability and the presence of PXXP and GGX motives within IDLs will be inspected.

Results: SSB protein sequence analyses suggested a correlation between amino aa composition and GC content. We will show how two distinct domains of SSB exhibit significant difference to the expected aa composition. In line with this, we will discuss the conservation of PXXP and GGX motives in SSBs within analysed bacterial phyla and with the respect to GC content.

PT084 An integrative vector for surface display expression of large nanobody libraries inserted in the chromosome of E.coli

Lidia Cerdán[1], Beatriz Álvarez[1], Luis Angel Fernandez[1]

[1]*National Center for Biotechnology, Microbial Biotechnology, Madrid, Spain*

Background: Nanobodies (Nbs) are single domain antibodies from camelid heavy-chain-only antibodies (HCAbs). Nbs are attractive molecules for human therapy and *in vivo* diagnostics. We have previously developed a multicopy plasmid system for the surface display in *E. coli* of Nb libraries fused to Intimin N-fragment (Neae) allowing selection of high affinity Nbs against antigens of interest.

Objectives: To develop a vector system for integration of Nb libraries as single copy in the chromosome of *E. coli* enabling surface display and selection of high affinity binders.

Methods: We employed a suicide plasmid with thermosensitive (ts) origin of replication for integration in the chromosome of *E. coli*. Genetic markers were used to isolated bacteria with integrated gene fusions. Surface display expression was analyzed by fluorescence flow cytometry and antigen-binding clones were selected by magnetic cell sorting (MACS) and fluorescent activated cell sorting (FACS).

Results: We demonstrate the efficient and site-specific integration of large libraries of Neae-Nb fusions in the chromosome of *E. coli* using our suicide ts-plasmid vector and integration strategy. Surface display of Neae-Nb fusions from single-copy expression was shown by flow cytometry. High affinity antigen-binding clones from the integrated library were selected against human Epidermal Growth Factor Receptor (EGFR). In conclusion, our system allows the integration of large Nb libraries in the chromosome of *E. coli* for surface display and selection. This system has the potential to be used with other large gene libraries (e.g. antibodies, enzymes, etc.) for their integration the chromosome of *E. coli*.

PT085 Development of Streptomyces strains to utilise sustainable feedstocks in fermentations

Anna Birke[1], Steve Kendrew[2], Benjamin Huckle[2], Iain Hunter[1], Paul Hoskisson[1]

[1]*University of Strathclyde, Strathclyde Institute of Pharmacy and Biomedical Sciences, Glasgow, United Kingdom*
[2]*GlaxoSmithKline, Worthing, United Kingdom*

Background: *Streptomyces clavuligerus* is used by GlaxoSmithKline for the industrial production of the β-lactamase inhibitor clavulanic acid. In fermentations, the carbon source available to the producing organism has a profound impact on central carbon and specialised metabolism. Wildtype *S. clavuligerus* has a narrow carbon utilisation profile and is a natural glucose auxotroph. Previous studies have shown the lack of glucose utilisation in *S. clavuligerus* is due to insufficient expression of the glucose permease (*glcP*) and kinase (*glk*) genes.

Objectives: The goal is to use carbon extracted from food waste in fermentations to render the production of clavulanic acid sustainable. Therefore, the overall carbon utilisation profile and enzymatic capabilities of *S. clavuligerus* must be increased. Furthermore, existing metabolic links between clavulanic acid biosynthesis and carbon utilisation, as well as regulatory mechanisms thereof must be better understood.

Methods: Clavulanic acid production strains were constructed for heterologous expression of either a *glcP* or *glk* gene taken from a *Streptomyces* species. The constructed strains are analysed in terms of their growth, developmental phenotype and clavulanic acid production in the presence of glucose or other carbon sources.

Results: For heterologous expression of the *glcP* and *glk* genes, integrating vectors were constructed with the genes of interest placed under the control of a thiostrepton-inducible promotor. Carrying out growth curves of the constructed strains was optimised to allow rapid identification of a strain's ability to utilise glucose in liquid medium. Extracellular protease production has been established as a proxy for carbon utilisation on solid medium.

PT086 Characterization of cyberlindnera jadinii carboxylate permeases by heterologous expression in saccharomyces cerevisiae

Maria Antónia Sousa-Silva[1;2], David Ribas[1;2], Margarida Casal[1;2], Isabel Soares-Silva[1;2]

[1]*Institute of Science and Innovation for Bio-Sustainability (IB-S), University of Minho, Braga, Portugal*
[2]*CBMA - UMinho, Biology Department, Braga, Portugal*

Background: The wide applicability of organic acids for direct use as commodity chemicals and as polymer building blocks has evidenced their importance in diverse types of industries. In *Saccharomyces cerevisiae,* two permeases are responsible for the uptake of carboxylates (CA) at the plasma membrane, Jen1p a monocarboxylate proton symporter (Major Facilitator Superfamily) and Ady2p an acetate permease (AceTr Family).

Objectives: In *Cyberlindnera jadinii*, different uptake systems for CAs were functionally characterized however until now the genes encoding these transporters remain unidentified. In this study, CA transporter homolog genes from *C. jadinii* were identified and expressed in *S. cerevisiae*.

Methods: The *S. cerevisiae* strain W303-1A *jen1*Δ *ady2*Δ, lacking carboxylate uptake capacity, was used to express *C. jadinii ScADY2* homologs. Genes were identified through sequence alignment and homology prediction and cloned in the p416GPD vector, under the control of a GPD constitutive promoter. GFP-fusions versions were used to determine protein expression and localization. Transport activity was determined through growth on different carbon sources and measurement of the uptake of labelled CAs, namely D,L-[U-^{14}C] lactic acid, [2,3-^{14}C] succinic acid and [1-^{14}C] acetic acid.

Results: In *C. jadinii*, 4 genes homolog to *ScADY2* were identified. These are functional carboxylate transporters in *S. cerevisiae,* localized at the plasma membrane*,* presenting different specificities for the mono- and di-carboxylates. Further studies are underway to fully characterize these four new plasma membrane transporters, including molecular docking of these transporters to unveil the amino acids that play a major role in the substrate binding of CAs tested.

PT087 Ethanol production from cassava starch using palm wine yeasts immobilized on wheat-gluten

Tolulope Adeleye[1], Sarafadeen Kareem[1]

[1]*Federal University of Agriculture Abeokuta, Microbiology, Abeokuta, Nigeria*

Background: Cell immobilization ahs been identified as a technique for improving ethanol production during yeasts fermentation. However, commercially available supports remain cost in-effective for a growing economy.

Objectives: The study was conducted to determine the effect of wheat-gluten as a support for the immobilization of yeasts from palm wine, on ethanol production from cassava starch.

Methods: Thirty one yeasts were isolated from fresh palm wine obtained from different locations in Abeokuta, Nigeria and screened for their fermentative abilities. Further screening was carried out in cassava starch hydrolyzed with commercial amylases at 4% (w/v) (pH 6.0, 80oC) and saccharified with glucoamylase at 4% (w/v) (pH 4.5, 600C). Analyses of volumetric ethanol productivity (Q), carbon dioxide productivity (QCO2) and ethanol tolerance of yeast isolates were carried out using standard chemical methods. Suspended cells and cells immobilized on gluten beads (1.0cm and 1.5cm) of selected isolate designate T01 were used for further comparative studies.

Results: There was no significant difference ($p>0.05$) in ethanol production using T01 immobilized on 1.0cm bead sizes and free cells. However, cells immobilized on gluten pellets of 1.5cm gave a 30% increase in fermentation efficiency and improved ethanol production over free cells. While a decline in fermentation parameters, was obtained during repeated batch fermentation using free cells, no significant decline (at $p>0.05$) was observed using cells immobilized on gluten beads (1.5cm). This study presents cheap agricultural material such as wheat-gluten as a re-usable and efficient support for cell immobilization for ethabol production from fermented starch hydrolysate.

PT089 Escherichia coli-based cell-free synthesis of the sweet-tasting protein, brazzein

Illia Kazlouski[1], Anatoly Zinchenko[1]

[1]*The Institute of Microbiology of NAS of Belarus, Laboratory of Molecular Biotechnology, Minsk, Belarus*

Background: The global increase of obesity and diabetes mellitus has led to an explosive interest for healthy natural sweeteners with favorable tasting properties. Sweet-tasting proteins may constitute an interesting group of sucrose. Brazzein is the smallest sweet-tasting protein with a taste close to that of natural sugars. In addition, brazzein presents physicochemical characteristics such as high water-solubility and extreme thermostability, which are essential for food applications. Synthesis of brazzein in whole cell expression systems has one main limitation, namely more than 70 % of the protein is produced within inclusion bodies, requiring chemical refolding steps.

Objectives: We propose *Escherichia coli*-based cell-free protein synthesis (CFPS) as an excellent bioprocess technology for the production of brazzein. Also of interest when producing brazzein is the ability of CFPS to produce high yields of proteins that are often cytotoxic or insoluble *in vivo*.

Methods: We used the classical techniques of genetic engineering to construct plasmid and conducted CFPS reaction for obtaining high yields of brazzein.

Results: We have demonstrated that *E. coli*-based CFPS can produce brazzein at yields of 1.5 mg/mL, which is over 10 times greater than the best previously reported yields using whole cell expression systems. Characterization of brazzein by gel-electrophoresis, thermo- and pH stability tests verified that it is similar to previous *in vivo* produced brazzein. To our knowledge, this is the first time a sweet-tasting protein has been generated using a CFPS system. These results demonstrate the feasibility for this production technology to satisfy the demand for brazzein production technology.

PT090 Studying the metabolism of Streptomyces coelicolor during antibiotic production in submerged batch fermentations

Kanhaiya Kumar[1], Alexander Wentzel[2], Per Bruheim[1]

[1]*Norwegian University of Science and Technology, Department of Biotechnology and Food Science, Trondheim, Norway*
[2]*SINTEF Industry, Department of Biotechnology and Nanomedicine, Trondheim, Norway*

Background: The presented work relates to our efforts to develop optimized *Streptomyces* 'Superhost' strains for heterologous production of new bioactive compounds by using Systems and Synthetic Biology approaches. One important experimental input to this task is deciphering the participation of important metabolic pathways for antibiotic production and generation of high-resolution quantitative metabolite profiles of the *Streptomyces coelicolor* host production strains.

Objectives: To study the carbon distribution across metabolic pathways along with metabolite profiling during antibiotic production in *Streptomyces coelicolor* production host strains.

Methods: Intracellular metabolite pools were quantified using several MS/MS based methods; i.e. two UPLC-MS/MS methods for amino acids and organic acids, a capIC-MS/MS method for nucleotides, sugar phosphates and other phosphometabolites.

Results: Fermentation medium designed to provide either L-glutamate or phosphate limitation at a defined time during cultivation triggered antibiotics production in *Streptomyces coelicolor* A3(2). Contrary to phosphate limitation, L-glutamate limitation (as nitrogen limitation an/or depletion of the preferred carbon source) triggered the stringent stress response as observed by a sharp decline in the CO_2 production and biomass concentration. Phosphate limitation downregulated the sugar and nucleotide phosphate metabolites pools, whereas L-glutamate limitation was found to reduce the citric acid cycle metabolite pools. ^{13}C-isotope-labeling experiments showed the degree of participation of carbon from L-glutamate and D-glucose in antibiotics and their precursor compounds. The metabolite pools in the non-antibiotic producing *S. coelicolor* host strain were flooded with carbon from both carbon sources, whereas in antibiotic producing strains, the carbon of L-glutamate seems to be draining out through antibiotic synthesis.

PT091 Screening of cnidarian bacterial symbionts for the potential biosynthesis of NRPS bioactive compounds against pancreatic cancer

Mafalda Tabuada[1], Carlos Moura[2], Amadeu M.V.M. Soares[1], Catarina Marques[1]

[1]Universidade de Aveiro, Departamento de Biologia & CESAM (Centro de Estudos do Ambiente e do Mar), Portugal
[2]University of Azores, DOP, Inst. Mar IMAR, MARE Marine & Environmental Sciences Centre, Portugal

Background: The high malignancy of pancreatic cancer along with severe limitations of the current treatments, is forcing the search for new anticancer drugs from natural sources. Marine natural products have been isolated from different sources like microbes, algae, and cnidarians, being of particular biotechnological interest bacterial symbionts of marine invertebrates. Nonribosomal peptides (NRPs) are a group of natural compounds known by their remarkable structural diversity and varied bioactivities.

Objectives: The aim of this work was to apply a molecular approach to screen for the biosynthetic potential of bacterial symbionts of marine cnidarians, and to test the bioactivity of crude extracts of selected bacteria against a human pancreatic ductal adenocarcinoma cell line.

Methods: Bacteria were isolated from cnidarian individuals collected in the Atlantic Ocean. A conserved region of the nonribosomal peptide synthetase(NRPS) A domain was amplified by PCR and sequenced whenever high amplification was obtained. Bacteria harbouring NRPS gene fragments were identified through 16S rRNA sequencing, and the crude extracts (cell and cell-free fractions) were prepared by ethyl acetate and/or acetone extraction. The cytotoxicity of crude extracts was tested against Capan-1 cell line through the MTT assay.

Results: The NRPS gene fragments was amplified in 22% of the marine bacteria, which were assigned to the Proteobacteria and Actinobacteria phyla. Fourteen strains were selected for NRPS sequencing and extract preparation. High homology by consensus with NRPS, PKS or hybrid genes was obtained in 8 of them, although 96.4% of bacterial crude extracts were cytotoxic to Capan-1 human pancreatic ductal adenocarcinoma cell line.

PT092 Membrane engineering via trans-unsaturated fatty acids production increased succinic acid production in Mannheimia succiniciproducens

Jong An Lee[1], Jung Ho Ahn[1], Junho Bang[1], Sang Yup Lee[1]

[1]*KAIST, Department of Chemical and Biomolecular Engineering, Daejeon, Korea, Rep. of South*

Background: Engineering of microorganisms to produce desired bio-products with high titer, yield, and productivity is often limited by product toxicity. This is also true for succinic acid (SA), a four carbon dicarboxylic acid of industrial importance. Acid products often cause product toxicity to cells through several different factors, membrane damage being one of the primary factors.

Objectives: Development of an engineered *Mannheimia succiniciproducens* strain with improved SA tolerance for enhanced production of SA.

Methods: The membrane engineering strategy involves the expression of CTI and PelB signal peptide in the *M. succiniciproducens* PALK strain (*ldhA*$^{-}$, *pta*$^{-}$, *ackA*$^{-}$) to increase *trans*-unsaturated fatty acid (TUFA) content in the cell membrane. Strategy for producing longer chain fatty acids was also employed to synthesize a thicker membrane to further enhance succinic acid tolerance.

Results: The engineered strain significantly reduced membrane fluidity by increased TUFA concentration in the cell membrane, and consequently showed increased tolerance to SA and lower pH compared to the control strain. The SA titer, yield, and productivity obtained by fed-batch fermentation of the final PALK (pMS3-pelB-cti) strain were 84.21 g/L, 1.27 mol/mol glucose, and 3.20 g/L/h, respectively, when glucose was utilized as sole carbon source, showing higher values of all three performance indices by membrane engineering. Fed-batch culture of the PALK (pMS3-pelB-cti) strain using glucose and glycerol further increased SA production. This work was supported by the C1 Gas Refinery Program funded by the Ministry of Science and ICT through the National Research Foundation of Korea (NRF-2016M3D3A1A01913250).

PT093 Structural basis for the bacterial plastic, polyhydroxyalkanoate (PHA) biosynthesis

So Young Choi[1], Yeo-Jin Kim[2], Jieun Kim[2], Sang Yup Lee[1], Kyung-Jin Kim[2]

[1]Korea Advanced Institute of Science and Technology, Chemical and Biomolecular Engineering, Daejeon, Korea, Rep. of South
[2]Kyungpook National University, Korea, Rep. of South

Background: Polyhydroxyalkanoates (PHAs) are natural polyesters synthesized by numerous microorganisms as energy and reducing power storage materials, and have attracted much attention as substitutes for petroleum-based plastics. PHA synthase (PhaC) is the key polymerizing enzyme determining the characteristics of PHAs such as monomer composition and molecular weight. However, the detailed of molecular mechanisms of PhaC have not been fully understood due to the lack of structural information on PhaC.

Objectives: PHA biosynthesis mechanism was studied at a molecular-level based on the 3-dimensional (3D) structure of PhaC. It aimed to understand the PHA biosynthesis and establish the framework for the rational engineering of PhaC toward more efficient production of PHAs composed of desired monomers.

Methods: We obatined the crystal of *Cupriavidus necator* PhaC, one of the most widely studied PhaCs. From the crystal structure, the detailed mechanisms and characteriestics of PhaC were investigated through substrate docking simulation, site-directed mutagenesis, small angle x-ray scattering analysis, and related biochemical studies.

Results: We revealed the 3D structure of catalytic domain of *C. necator* PhaC with the possible substrate-binding mode and catalytic mechanism. We also constructed the 3D models of overall *C. necator* PhaC and the complex of PhaC with PhaM. Based on the models, the roles of each domain of PhaC have been suggested. [This work was supported by the Technology Development Program to Solve Climate Changes on Systems Metabolic Engineering for Biorefineries from the Ministry of Science, ICT and Future Planning (MSIP) through the National Research Foundation (NRF) of Korea (NRF-2012M1A2A2026556 and NRF-2012M1A2A2026557).]

PT094 Fermentative production of four-, five-, and six-carbon lactams via novel metabolic pathway

Tong Un Chae[1], Yoo Sung Ko[1], Kyu-Sang Hwang [1], Sang Yup Lee[1]

[1]Korea Advanced Institute of Science and Technology (KAIST), Chemical & Biomolecular Engineering, Daejeon, Korea, Rep. of South

Background: Bio-based production of chemicals and materials from renewable non-food biomass is a promising option to achieve sustainable chemical industry. Towards this goal, numerous microorganisms have been metabolically engineered for the bio-based production of various chemicals and materials. Among these chemicals, there also have been some reports on the bio-based production of various lactams which is widely used chemicals as monomer for polyamide. However, the production efficiency of lactams were rather low.

Objectives: Here, we report development of a new and efficient platform metabolic pathway for the fermentative production of three important lactams, butyrolactam, valerolactam and caprolactam.

Methods: This pathway uses ω-amino acids as precursors and comprises two steps. Activation of ω-amino acids catalyzed by the *Clostridium propionicum* β-alanine CoA transferase (Act) followed by spontaneous cyclization. The pathway operation was validated by both *in vitro* and *in vivo* assays. Furthermore, three metabolically engineered *Escherichia coli* strains were developed by introducing the newly constructed metabolic pathway followed by systems-level optimization, which resulted in the production of butyrolactam, valerolactam and caprolactam from renewable carbon source.

Results: In particular, fed-batch fermentation of the final engineered *E. coli* strain produced 54.14 g/L of butyrolactam in a glucose minimal medium. These results demonstrate the high efficiency of the novel lactam pathway developed in this study. [This work was supported by the Technology Development Program to Solve Climate Changes on Systems Metabolic Engineering for Biorefineries from the Ministry of Science, ICT and Future Planning (MSIP) through the National Research Foundation (NRF) of Korea (NRF-2012M1A2A2026556 and NRF-2012M1A2A2026557)]

PT095 The emerging opportunistic pathogen Abiotrophia defectiva- a case report and review

Shin-Yi Tsai[1;2], Yu-Ting Tina Wang[1], Yi- Chen Ho[1;2], Steven Ho[1;2], Chien- Feng Kao[3], Wei- Shieng Wang[3]

[1]*Mackay Memorial Hospital, Department of Laboratory Medicine, Taipei City, Taiwan*
[2]*Mackay Medical College, Department of Medicine, New Taipei City, Taiwan*
[3]*Mackay Memorial Hospital, Department of Infectious Diseases, Taipei City, Taiwan*

Background: The bacteria *Abiotrophia* spp. and *Granulicatella* spp. may be the responsible organism for 5 to 6% of all cases of streptococcal infective endocarditis (IE). This makes them under- reported, contributing to higher rates of complications: higher mortality, risk of embolization, treatment failure, compared with *Viridans streptococci*.

Objectives: Several diagnostic methods should be considered for early diagnosis and management to prevent complications.

Methods: A 55-year-old woman with no underlying disease visited the Thoracic Medicine clinic at MacKay Memorial Hospital complaining of intermittent right chest wall pain for several months. Physical examination showed redness, swelling, local heat, tenderness over her right foot and Janeway and Osler lesions over her hands and feet. Furthermore, a pan-systolic murmur was found. Fever up to 38.1 was noted. Lab tests reported leukocytosis, anemia, and elevated CRP level. Blood culture identified *Abiotrophia defectiva* and she was treated with Augmentin and gentamicin.

Transthoracic echocardiogram showed an echogenic mobile mass (1.4x0.8cm) attached on atrial side of anterior mitral leaflet.

After metagenomic analysis of the resected vegetation, we detected *Abiotrophia defectiva*, which is often associated with culture-negative IE due to its fastidious growth.

Due to the indication of large vegetation >10mm according to the AHA guidelines, she decided to transfer to National Taiwan Hospital University (NTHU) for surgery.

Results: Traditionally, blood culture is the main diagnostic method however, there are difficulties in culturing *Granulicatella* and *Abiotrophia* spp. Thus, newer diagnostics with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF) and 16S rRNA sequencing for definitive identification is recommended.

PT096 Microbial malonyl-CoA biosensor utilizing type III polyketide synthase

Seon Young Park[1;2], Dongsoo Yang[1;2], Won Jun Kim[1;2], Seung Min Yoo[1;3], Jong Hyun Choi[4], Shin Hee Ha[1], Mun Hee Lee[1;2], Sang Yup Lee[1;2;3;5]

[1]Korea Institute of Advanced Science and Technology, Chemical and Biomolecular Engineering, Daejeon, Korea, Rep. of South
[2]Korea Institute of Advanced Science and Technology, Systems Metabolic Engineering and Systems Healthcare Cross-Generation Collaborative Laboratory, Daejeon,
[3]Korea Institute of Advanced Science and Technology, BioProcess Engineering Research Center and BioInformatics Research Center, Daejeon, Korea, Rep. of South
[4]Korea Research Institute of Bioscience and Biotechnology, Applied Microbiology Research Center, Jeongeup,
[5]Technical University of Denmark, Novo Nordisk Foundation Center for Biosustainability, Kongens Lyngby, Denmark

Background: Malonyl-CoA is an important building block for many valuable natural products including polyketides and phenylpropanoids, therefore there have been lots of efforts to increase intracellular malonyl-CoA pool.

Objectives: In this study, we developed simple colorimetric malonyl-CoA biosensor which is applicable in three different bacteria: *Escherichia coli*, *Pseudomonas putida*, and *Corynebacterium glutamicum*, and coupled the developed biosensor with synthetic small RNA (sRNA) library screening.

Methods: RppA, a type III polyketide synthase which produces red-colored flaviolin from malonyl-CoA was developed as a malonyl-CoA biosensor. After construction of the biosensor system, a 1,858 synthetic sRNA library was tranformed into *E. coli* to find knockdown target genes which enhanced intracellular level of malonyl-CoA. The selected sRNAs harboring knockdown target genes were applied to two polyketides and two phenylpropanoids producing *E. coli* strains, respectively.

Results: The developed RppA biosensor could respond to broad range of intracellular malonyl-CoA concentration in *E. coli*. As a result of synthetic sRNA library screening, 14 knockdown gene targets were selected. Application of the selected knockdown gene targets in four different *E. coli* strains producing 6-methylsalicylic acid, aloesone, resveratrol and naringenin, respectively, enhanced production of each compound up to 440.3, 30.9, 51.8, and 103.8 mg/L, respectively. [This work was supported by the Technology Development Program to Solve Climate Changes on Systems Metabolic Engineering for Biorefineries (Grants NRF-2012M1A2A2026556 and NRF-2012M1A2A2026557) and by the Intelligent Synthetic Biology Center through the Global Frontier Project (Grant 2011-0031963) of the Ministry of Science and ICT (MSIT) through the National Research Foundation of Korea.]

PT097 Assimilation of CO2 and formic acid by engineered Escherichia coli equipped with reconstructed one-carbon assimilation pathways

Jong An Lee[1], Junho Bang[1], Sang Yup Lee[1]

[1]*KAIST, Department of Chemical and Biomolecular Engineering, Daejeon, Korea, Rep. of South*

Background: Gaseous one-carbon (C1) compounds such as CO_2 and CH_4 are major greenhouse gases, which are largely responsible for global warming and climate change. With the aim of reducing greenhouse gases, biological conversion of C1 compounds has attracted much attention due to several advantages such as low energy requirements, environmental friendliness, and the possibility of converting greenhouse gases directly to value-added products.

Objectives: Allowing *Escherichia coli* to utilize C1 compounds (formic acid (FA) and CO_2) efficiently by combined use of the C1 assimilation pathway and formate dehydrogenase.

Methods: The *Methylobacterium extorquens* formate-THF ligase, methenyl-THF cyclohydrolase, and methylene-THF dehydrogenase genes were expressed to allow FA assimilation. The gcv reaction was reversed by knocking out the repressor gene (*gcvR*) and overexpressing the *gcvTHP* genes. The pyruvate-forming flux from FA and CO_2 could be increased to 14.9% by knocking out *gcvR*, *pflB*, and *serA*, chromosomally expressing *gcvTHP* under *trc*, and overexpressing the reconstructed THF cycle, *gcvTHP*, and *lpd* genes in one vector. To reduce glucose usage required for energy and redox generation, the *Candida boidinii* formate dehydrogenase (Fdh) gene was expressed.

Results: Engineered *E. coli* strains capable of efficiently utilizing FA and CO_2 by introducing the rTHF cycle and reverse gcv pathway is developed. The possibility of reducing the amount of glucose needed was also demonstrated by establishing the Fdh reaction. This work was supported by the C1 Gas Refinery Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science and ICT Grant NRF-2016M3D3A1A01913250.

PT098 Bioproduction of Poly(lactate-co-glycolate) [PLGA] by Metabolically Engineered Microorganism

So Young Choi[1], Won Jun Kim[1], Seung Jung Yu[1], Si Jae Park[2], Sung Gap Im[1], Sang Yup Lee[1]

[1]Korea Advanced Institute of Science and Technology, Chemical and Biomolecular Engineering, Daejeon, Korea, Rep. of South
[2]Ewha Womans University, Division of Chemical Engineering and Materials Science, Seoul, Korea, Rep. of South

Background: The biorefinery technologies which transform biomass into fuel, power, chemicals, and materials have received a great deal of attention as a sustainable alternative to decreasing the reliance on fossil fuels. Poly(lactate-co-glycolate) [PLGA] is a biodegradable, biocompatible and FDA-approved bioplastic which has been widely used in biomedical and therapeutic applications such as drug delivery and tissue engineering.

Objectives: We developed recombinant *Escherichia coli* strain producing PLGA and its copolymers from renewable non-food biomass to substitute the current chemical production process.

Methods: We engineered *E. coli* to efficiently produce two monomers, lactate and glycolate by introducing the Dahms pathway of *Caulobacter crescentus* and by modulating the metabolic fluxes based on the genome-wide *E. coli* model simulation. Then, the two engineered heterologous enzymes, propionyl-CoA transferase and polyhydroxyalkanoate synthase were expressed in the engineered *E. coli* strain to convert lactate and glycolate to lactyl-CoA and glycolyl-CoA, respectively and finally PLGA.

Results: The engineered *E. coli* strains produced PLGA and poly(lactate-*co*-glycolate-*co*-2-hydroxybutyrate) from xylose. By fed-batch cultivation, the *E. coli* strains produced PLGA copolymers contained 8.8 – 60.9 mol% of glycolate up to 6.93 g/L. In addition, the biocompatibility of 2-hydroxybutyrate containing polymers was examined by live/dead assay using human mesenchymal stem cells. It demonstrated the possibility of this non-natural polymers as new biomedical plastics.
[This work was supported by the Technology Development Program to Solve Climate Changes on Systems Metabolic Engineering for Biorefineries from the Ministry of Science, ICT and Future Planning (MSIP) through the National Research Foundation (NRF) of Korea (NRF-2012M1A2A2026556 and NRF-2012M1A2A2026557)].

PT099 Metabolic engineering of Escherichia coli for efficient production of ethylene glycol from xylose

Tong Un Chae[1], So Young Choi[1], Jae Yong Ryu[1], Sang Yup Lee[1]

[1]Korea Advanced Institute of Science and Technology (KAIST), Chemical & Biomolecular Engineering, Daejeon, Korea, Rep. of South

Background: Ethylene glycol (EG) is an important platform chemical, which is widely used as an antifreezing agent, film, and as a precursor of the most common plastic, poly(ethylene terephthalate). Currently, EG is mainly produced by chemical synthesis. As demand for sustainable chemical production increases, several engineered microbes have been developed for EG production from renewable carbon sources. However, production titer, yield and productivity need to be further improved for further commericial industrial-scale production.

Objectives: In this study, *Escherichia coli* was metabolically engineered to efficiently produce EG from xylose.

Methods: To biosynthesize EG, the Dahms pathway was introduced by expressing *xylBC* genes from *Caulobacter crescentus* ($xylBC_{ccs}$). Various *E. coli* strains and glycolaldehyde reductases were screened to find *E. coli* W3110 strain and glycolaldehyde reductase (*yqhD*) as optimal combination for EG production. *In silico* genome-scale metabolic simulation suggested that increasing the native xylose pathway flux, in the presence of the overexpressed Dahms pathway, is beneficial for EG production. This was achieved by reducing the Dahms pathway flux by employing a synthetic small regulatory RNA targeting $xylB_{ccs}$.

Results: Fed-batch culture of the final engineered *E. coli* strain produced 108.2 g/L of EG in a xylose minimal medium. The yield on xylose and EG productivity were 0.36 g/g (0.87 mol/mol) and 2.25 g/L/h, respectively. [This work was supported by the Technology Development Program to Solve Climate Changes on Systems Metabolic Engineering for Biorefineries from the Ministry of Science and ICT through the National Research Foundation (NRF) of Korea (NRF-2012M1A2A2026556 and NRF-2012M1A2A2026557)]

PT100 Gene expression level modulation by fine-tuning synthetic sRNA expression level for putrescine and L-proline overproduction in Escherichia coli

Seon Young Park[1], Minho Noh[1;2], Seung Min Yoo[1;2;3], Won Jun Kim[1;2], Sang Yup Lee[1;2;3]

[1]Korea Institute of Advanced Science and Technology, Chemical and Biomolecular Engineering, Daejeon,
[2]Korea Institute of Advanced Science and Technology, Institute for the BioCentury, Daejeon,
[3]Korea Institute of Advanced Science and Technology, BioProcess Engineering Research Center and Bioinformatics Research Center, Daejeon,

Background: Fine-tuning expression levels of the target genes is important for optimizing metabolic flux to minimize intermediates accumulation and to enhance product formation. Therefore, diverse strategies for diversifying gene expression level such as mRNA and protein stability regulation have been developed, however, these are inefficient for regulating chromosomal genes.

Objectives: To overcome the limitations of previous systems, we developed gene expression level fine-tuning system which can modulate expression levels of the target genes in mRNA level in *Escherichia coli*.

Methods: The previous developed synthetic small RNA (sRNA) system was utilized to efficiently knockdown expression levels of target genes in mRNA level. In order to fine-tune target gene expression level, expression level of corresponding sRNA was modulated by using different promoters.

Results: By using fine-tuned sRNA system, mRNA translation level of DsRed2 encoding gene could be simply and efficiently modulated. For enhanced putrescine production in *E. coli*, expression levels of 15 identified target genes were regulated, and the resulting strain exploiting fine-tuned repression of *argF* and *glnA* produced 42.3 g/L of putrescine. In L-proline producing *E. coli*, same gene target expression levels were differently modulated, and 33.8 g/L of L-proline was produced. [This work was supported by grants from the Technology Development Program to Solve Climate Change on Systems Metabolic Engineering for Biorefineries from the Ministry of Science and ICT (MSIT) through the National Research Foundation (NRF) of Korea (NRF-2012M1A2A2026556 and NRF-2012M1A2A2026557) and the Intelligent Synthetic Biology Center through the Global Frontier Project (2011-0031963) of MSIT through the NRF of Korea.]

PT101 Biosynthesis of Various Non-natural Aliphatic Polyesters in Microorganism

So Young Choi[1], Si Jae Park[2], Jung Eun Yang[3], Jihoon Shin[4], Sang Yup Lee[1]

[1]Korea Advanced Institute of Science and Technology, Chemical and Biomolecular Engineering, Daejeon, Korea, Rep. of South
[2]Ewha Womans University, Division of Chemical Engineering and Materials Science, Seoul, Korea, Rep. of South
[3]Korea Food Research Institute, Korea, Rep. of South
[4]Korea Research Institute of Chemical Technology, Korea, Rep. of South

Background: Polyhydroxyalkanoates (PHAs) are biodegradable polyesters accumulated in several microorganism. Recently, the PHA biosynthesis was applied to develop recombinant *Escherichia coli* strain producing a non-natural polyester, poly(lactate-*co*-glycolate) [PLGA] which has been widely used in biomedical and therapeutic applications such as drug delivery and tissue engineering.

Objectives: To develop a platform microbial strain producing various valuable non-natural polyesters, the PLGA producing *E. coli* strain was further engineered to produce various PLGA based copolymers by incorporating the natural and non-natural monomers with lactate and glycolate *in vivo*.

Methods: We exmained whether the metabolic pathway comprising of engineered propionyl-CoA-transferase and PHA synthase can be expanded to other hydroxyacids such as 3-hydroxybutyrate (3HB), 4-hydroxybutyrate (4HB), 5-hydroxyvalerate (5HV), and 6-hydroxyhexanoate (6HHA). Among the possible monomers, 3HB and 4HB were to be synthesized in vivo without any supplementation of precursors by constructing the biosynthetic pathways for 3HB-CoA and 4HB-CoA, respectively.

Results: The recombinant *E. coli* produced various PLGA based copolymers containing 2-hydroxybutyrate, 2-hydroxyisovalerate, 3HB, 4HB, 5HV or 6HHA. These copolymers showed enhanced polymer properties such as molecular weight, thermal and mechanical properties compared to those of PLGA. By further strain engineering, the *E. coli* strains producing poly(lactate-*co*-glycolate-*co*-4HB)s with various mole fractions of 4HB were developed. [This work was supported by the Technology Development Program to Solve Climate Changes on Systems Metabolic Engineering for Biorefineries from the Ministry of Science, ICT and Future Planning (MSIP) through the National Research Foundation (NRF) of Korea (NRF-2012M1A2A2026556 and NRF-2012M1A2A2026557)].

PT102 De novo production of 2-pyrone-4,6-dicarboxylic acid in metabolically engineered Escherichia coli

Seon Young Park[1;2], Zi Wei Luo[1;2], Won Jun Kim[1;2], Sang Yup Lee[1;2;3]

[1]Korea Institute of Advanced Science and Technology, Chemical and Biomolecular Engineering, Daejeon, Korea, Rep. of South
[2]Korea Institute of Advanced Science and Technology, Systems Metabolic Engineering and Systems Healthcare Cross-Generation Collaborative Laboratory, Daejeon, Korea, Rep. of South
[3]Korea Institute of Advanced Science and Technology, BioProcess Engineering Research Center and BioInformatics Research Center, Daejeon, Korea, Rep. of South

Background: 2-Pyrone-4,6-dicarboxylic acid (PDC) is a natural compound produced by *Sphingobium* sp. SYK-6, which can be utilized as a platform chemical for the synthesis of various polyesters. PDC-derived polymers are thermally stable and easily degraded. The chemical method for PDC synthesis has never been reported.

Objectives: In this study, we developed *Escherichia coli* strain that can efficiently produce PDC from glucose, by applying diverse metabolic engineering strategies.

Methods: Two different PDC biosynthetic pathways were compared via *in silico* simulation, and feedback repressions of the key enzymes were eliminated. PDC production was further enhanced by engineering an importer and increasing precursors availability. Then, the scale-up PDC production was carried out using engineered strain.

Results: The PDC biosynthetic pathway was constructed by exploiting single-step route toward protoxatechuic acid and introducing downstream genes in *E. coli*. The overexpression of feedback-resistant AroG variant and increasing expression level of native *ppsA* gene improved PDC production up to 2.28 g/L in flask culture. The overexpression of *E. coli shiA* for 3-dehydroshikimate import enhanced the titer up to 2. 41 g/L. Ultimately, fed-batch fermentation of the final strain resulted in 16.72 g/L of PDC production which is the highest value reported to date. [This work was supported by the Intelligent Synthetic Biology Center through the Global Frontier Project (2011-0031963) and also by the Technology Development Program to Solve Climate Changes on Systems Metabolic Engineering for Biorefineries (NRF-2012M1A2A2026556 and NRF-2012M1A2A2026557) from the Ministry of Science and ICT through the National Research Foundation of Korea.]

PT103 Lipids - fundamental eleemnts in the evolution and maintenance of cellular systems

Brian J. Tindall[1]

[1]*Leibniz-Institut DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH., Braunschweig, Germany*

Biology is ultimately based on chemistry and physics. The most fundamental building blocks of life start at the level of C1 and C2 compounds.

Further elaboration of these basic units leads to the synthesis of sugars, amino acids and lipids, with nucleic acids being derived from amino acid and sugar synthesis. Sugars, amino acids and nucleic acids are defined by their chemical structures, while lipids are defined primarily based on their physical properties.

Examining the chemical nature of lipids indicates that there are two distinct chemical groups – those based on isoprenoids (multiples of C5) and those based on fatty acids (multiples of C2), although the primary physical property of these two distinct groups are their hydrophilic or amphiphilic nature. It is their hydrophilic or amphiphilic nature that has led to the evolution of an ingenious biological structure, the cell membrane, without which life as we know it would not exist.

Despite their fundamental role in making the cell membrane (and life) possible the diversity in lipid structures and their significance in the biology of modern prokaryotes is largely neglected. Appreciating the diversity of lipid structures, together with their underlying biosynthetic pathways and encoding genes is a critical step in appreciating the role of this highly diverse range of small molecules (molecular weights of typically less than 2000). Understanding their chemical and physical properties and their interaction with other components of the cell membrane is critical to understanding the functioning of this, the largest and most complex part of the cell.

PT104 Bioethanol production from brown seaweeds and aquatic macrophytes

Ftepti Jelani[1], Joseph Akunna[2], Graeme Walker[3]

[1]Abertay University, school of science, engineering and technology, dundee, United Kingdom
[2]Abertay University, urban water technology centre, school of science, engineering and technology, dundee, United Kingdom
[3]Abertay University, yeast research group, school of science, engineering and technology, dundee, United Kingdom

Background: The use of Aquatic biomass as potential sources for biofuels production has attracted significant attention worldwide. This is mainly due to the fact that both marine and fresh water species, especially seaweeds, water hyacinth and water lettuce contain rich sources of polysaccharides coupled with high biomass production rate. They also lack competition with food crops. Therefore, seaweeds and aquatic macrophytes represent excellent sustainable choices for microbial conversion to ethanol.

Objectives:

1. To investigate the potential of seaweeds and aquatic macrophytes to produce ethanol.
2. To investigate the ability of different yeast strains to ferment a wide range of sugars found in seaweeds and aquatic macrophytes.

Methods: In the present study, the separate hydrolysis and fermentation method (SHF) was used to determine the potential of these plants for ethanol production. Fermentations were carried out using three yeast strains, *Saccharomyces cerevisiae*, *Pichia stipitis (*aka *Scheffersomyces stipitis) and Kluyveromyces marxianus.*

Results: *Pichia stipitis* yeast strain showed high affinity to ferment the sugar rhamnose which is the dominant in seaweed hydrolysate to produce 5.9 g/L, 2.8 g/L and 1.8g/L ethanol in *Laminaria digitata*, *Ascophyllum nodosum* and *Sargassum fluitans* respectively. In addition, Saccharomyces *cerevisiae* yeast strain showed high affinity to ferment glucose, which is dominant in the aquatic macrophyte hydrolysates to produce 4.7g/L and 4.1 g/L ethanol in *Eichhornia crassipes* and *Pistia stratiotes* respectively. These research findings demonstrate that aquatic biomass can be subject to microbial conversion to ethanol in relatively straightforward bioprocesses.

PT105 Bioprocess design - From small scale biology to large scale production.

Ellis Robb[1], Alison Arnold[1]

[1]*Ingenza Ltd, Fermentation Department, Edinburgh, United Kingdom*

Background: Ingenza – a leading UK Industrial Biotechnology company develops novel bioprocesses to manufacture valuable industrial products at a large scale. This requires expertise at all points throughout the roadmap of a project. From rapid and precise strain development through to calculated bioprocess design and optimisation each step is required to be in sync with the final goal.

Objectives: This poster will outline how the fermentation department at Ingenza is central to this strategy. It will highlight the importance of retrofitting the research to meet the requirements of the envisioned bioprocess at a large scale. Our team aim to carefully consider each aspect of the process during its development, in order to overcome the inherent challenges attributed to production at this scale.

Methods: With our expertise in using a wide range of host microorganisms, proven scalable fermentation methods and a targeted approach to process optimization, we can clearly demonstrate the feasibility of a project.

Results: This poster will give some real industrial examples of bioprocesses developed at Ingenza using our understanding of fermentation development and scale up. This knowledge is complemented by our core competencies in combinatorial genetics (inABLE®), protein engineering, solid phase screening/selection methods and precision analytical chemistry. Through these Ingenza combines consolidated technologies with new approaches at the forefront of innovation to drive small scale biology through to large scale production.

PT106 Genetic Modules to Use Enforced ATP-Wasting as a Tool for Metabolic Engineering

Ahmed Zahoor[1], Simon Boecker[1], Steffen Klamt[1]

[1]*MPI Magdeburg, Magdeburg, Germany*

Background: ATP-wasting has been proposed as a tool to improve the productivity and yield of desired metabolites by cell factories [1]. The concept consists of two steps: First, a knock-out strategy is established to make the (by)production of a target metabolite mandatory for net ATP synthesis. This is followed by enforced ATP wasting (e.g. by overexpression of ATPases or futile cycles), thereby forcing the cells to increase ATP synthesis to keep up with the cellular maintenance demand and simultaneously leading to higher specific productivity and yield.

Objectives: Providing a proof-of-principle for the use of enforced ATP wasting to improve production performance of microbial strains for synthesis of ATP-coupled products.

Methods: Inducible expression of the cytosolic F_1-subunit of the *E. coli* ATPase (catalyzing the hydrolysis of ATP to ADP and P_i) in wild type *E. coli* and *S. cerevisiae* strains and analyzing its influence on the fermentative product formation under anaerobic growth conditions.

Results: Overexpression of the ATPase resulted in approx. 10% increase in titer, yield and specific productivities of *E. coli* fermentation products. However, as growth rates decreased, the volumetric productivity dropped compared to the wild type strain. This issue was addressed by applying a two-stage-process, where growth and production phases are decoupled [2]. With this approach, the volumetric productivity could be doubled during the production phase, making this concept interesting for industrial applications.

[1] Hädicke et al., Biochem Soc Trans 2015, 43, 1140-1145

[2] Klamt et al., Biotechnol J 2018, 13, 1700539

PT107 Glutamate biosensor with two component regulatory system in Escherichia coli

Jong-il Choi[1], Sung-Ho Park[1]

[1]*Chonnam National University, Department of Biotechnology and Bioengineering, Gwangju, Korea, Rep. of South*

Background: Nature has provided numerous specific molecular biosensors that employ different transcriptional regulatory mechanisms in bacteria. Among these, the two-component regulatory system (TCRS) is the main signal transduction mechanism in bacteria, which regulates transcription through a sensor histidine kinase (SK) and response regulator (RR). Therefore, the use of a TCRS or chimeric TCRS as a biosensor to correlate the detection of a target molecule with the expression of a reporter protein is a promising methods that can be used in developing microbial cell factories.

Objectives: The aim of this study was to develop chimeric TCRS-based biosensors for glutamate-overproducing strains and detect key intermediates, specifically glutamate.

Methods: The DegS, a sensor kinase localized at the cytoplasm due to the absence of hydrophobic domain,was also identified to sense glutamate with high specificity. Careful analysis of the Pfam domain database revealed that DegS-DegU TCRS is also present in the genome of *Planococcus sp.* PAMC21323(GenBank accession no. CP009129). Thus, we took advantage of this newly identified system to engineer a chimeric DegSZ TCRS by fusing sensor histidine kinase DegS from PAMC21323 with the catalytic domain EnvZ from *E. coli* to generate a biosensor for glutamate detection.

Results: the linear detection range of the synthetic TCRS-based biosensor was 0–2.0 mM glutamate, with saturation occurring between 2.0 and 4.0 mM. This biosensor also allowed the use of FACS to identify cells producing glutamate for high-throughput screening when determining the novel fine-tuned metabolic pathways for the robust and efficient production of target molecules.

PT108 Generation of stable influenza virus hemagglutinin through structure-guided block swapping

Yu-Chan Chao Yc[1], Chih-Hsuan Tsai[1]

[1]*Academia Sinica, Institute of Molecular Biology, Taipei, Taiwan*

Background: Hemagglutinin (HA) is the major surface antigen of influenza virus and the most promising influenza vaccine immunogen. However, the HA of many of these viruses is relatively unstable, making it challenging to produce effective vaccine.

Objectives: Here, we applied a novel structure-guided bioinformatics software, SCHEMA, to rationally design chimeric HA, for improving HA protein stability from the A/Anhui/1/2013 (H7N9) viral strain.

Methods: To actively improve the stability of HA protein from H7N9 influenza virus for better vaccine antigens without impairing immunogenicity, we recombined the HA from H7N9 (H7) with a more stable HA from H3N2 (H3) by structure-guided block swapping.

Results: The structure-guided block swapping resulted in six chimeric HAs, fC1 to fC6. Two of these chimeric HAs, fC2 and fC3, exhibited proper hemagglutination activity and presented improved thermal stability compared to the original H7. Mice immunized with fC2 and fC3 elicited H7-specific antibodies comparable to those induced by parental H7, and the antisera collected from these immunized mice successfully inhibited H7N9 infection in a micro-neutralization assay. These results suggest that our structural-recombination approach can create stabilizing chimeric antigens while maintaining proper immunogenicity, which may not only benefit the construction of more stable HA vaccines to fight against H7N9 infection, but also facilitate effective vaccine improvements for other influenza viruses or infectious pathogens. In addition, this study also demonstrates the potential for better engineering of multimeric protein complexes like HA to achieve improved functionality, which are often immunologically or pharmaceutically important but difficult to modify.

PT109 Expression, Purification and Derivatization of the Recombinant Protein Lsa45 of Leptospira interrogans

Rebeca Cordellini Emidio[1,2], Jademilson Celestino Santos[1], Luis Guilherme Virgílio Fernandes[1], Ana Lucia Tabet Oller do Nascimento[1], Giovana Barazzone[1]

[1]*Instituto Butantan, Laboratório Especial de Desenvolvimento de Vacinas, Sao Paulo, Brazil*
[2]*Universidade de São Paulo, Instituto de Ciências Biomédicas, Sao Paulo, Brazil*

Background: Leptospirosis is a worldwide emergent disease. *Leptospira* features pathogenic and saprophytic species, and lipopolysaccharide (LPS) is claimed responsible for its antigenic diversity. *Leptospira* surface proteins capable of binding to extracellular matrix (ECM), a fundamental step in the infection, are described in the literature. One of them is Lsa45, which interacts with ECM's laminin and plasminogen generating active plasmin, able to induce humoral and cellular immunological response in mice.

Objectives: To find the best rLsa45 expression condition and derivatization efficiency for further chemical conjugation with *L. biflexa* LPS.

Methods: *E. coli* BL21 pGro7 cells were transformed with pAE and pET28a/Lsa45 expression vectors and stir-cultivated at 37°C in Erlenmeyer (ER) and Tunair (TA), in LB and self-induction (SI) media with antibiotic, until OD reached 0.6-0.8, induced by 1mM IPTG, and glucose depletion, respectively. Then, cells were harvested by centrifugation, resuspended and disrupted. Supernatant was loaded into a nickel affinity column, followed by size exclusion chromatography (SEC). Purified rLsa45 was reacted with adipic acid dihydrazide (ADH) and purified by SEC. Amine and protein contents were evaluated.

Results: Using pAE, TA-SI culture yielded 19.43 mg/L of purified protein (30% recovery), TA-LB yielded 8.85 mg/L (31% recovery), ER-SI, 7.29 mg/L (35% recovery) and ER-LB, 2.06 mg/L (31% recovery). With pET28a vector, TA-SI yielded 15.44 mg/L (29% recovery) and ER-SI, 4.80 mg/L (31% recovery). After derivatization, the molar ratio between -NH_2 and rLsa45 was 13. The final amine content was thrice the initial one, aiding the conjugation between rLsa45 and *L. biflexa* LPS.

PT110 enhanced production of poly-3-hydroxybutyrate (phb) by expression of response regulator dr1558 in recombinant escherichia coli

Sungho Park[1], Jong-il Choi[1]

[1]Chonnam National University, Biotechnology and Bioengineering, Interdisciplinary Program for Bioenergy & Biomaterials, Gwangju, Korea, Rep. of South

Background: Two-component signal transduction systems (TCS) that comprises of histidine kinase (HK) sensor and a response regulator (RR) are efficient in improving cell tolerance under stress conditions. *Deinococcus radiodurans* is known to be resistant to various abiotic stress factors. Response regulator DR1558 from *D. radiodurans* introduces alterations in the cell either directly, by binding to the promoter regions of genes via DNA-binding domain, or indirectly through effector molecules, leading to a cellular output against environmental stress.

Objectives: For the efficient production of PHB, many genes involved in metabolic pathway were expressed and modified. But, there was no previous study on the effect of stress-tolerant genes for PHB production. During the PHB fermentation, some byproducts were accumulated and inhibited cell viability. Therefore, the increase of stress-tolerance by introducing DR1558 could enhance the PHB production.

Methods: We examined effect of overexpression of DR1558 for the production of PHB in recombinant *E. coli* in the aspects of cell growth, glucose consumption, PHB production, and other metabolites accumulation. furthermore, the changes caused by DR1558 in transcriptional level were analyzed by comparing expression level of some important genes in *dr1558* overexpressing strains to the control by quantitative real-time PCR.

Results: Overexpression of *dr1558* could be suggested to enhance metabolic performance of PHB producing *E. coli* by improving expression efficiency of numerous genes related to central carbon metabolism. Especially, the metabolic changes such as acetate synthesis and utilization pathway in our results suggest that avoiding acetate accumulation together with increasing target product production.

PT111 Bioremediation of radioactive iodine using radiation-resistant bacterium, Deinococcus radiodurans R1

Jung Eun Yang[1], Yong Jun Choi[2]

[1]*World Institute of Kimchi, Advanced Process Technology and Fermentation, Gwangju, Korea, Rep. of South*
[2]*University of Seoul, School of Environmental Engineering, Seoul, Korea, Rep. of South*

Background: Due to the severe adverse effects of radioactive substances, development of sustainable treatment method for the removal of radioactive wastes is urgently needed for our public health. Among the radioactive wastes, after used radioactive iodine has been regarded as most hazardous contaminant as it has strong radioactivity triggering various chronic diseases. Recently, sustainable bioremediation strategies using microorganism have been reported. The radiation resistant bacterium *Deinococcus radiodurans* R1 is the best known for its remarkable resistance to external stresses such as high radiation, oxidants, and desiccation. Although *D. radiodurans* R1 strain has great potential, it has not yet been applied for the bioremediation of toxic contaminants.

Objectives: Development of sustainable bioremediation technology for the treatment of radioactive iodine using extremophilic microorganism, *Deinococcus radiodurans* R1.

Methods: To achieve this goal, *Deinococcus radiodurans* R1 strain was forced to synthesize gold nanoparticles inside the cells through biosorption process. Diverse morphologies and size of gold nanoparticles were efficiently synthesized inside the cells and confirmed by scanning electron microscopy (SEM) and Dark field microscopy analysis. The adsorption of radioactive iodine onto gold nanoparticle was confirmed by single photon emission computed tomography (SPECT) and computed tomography (CT) scanning.

Results: More than 3.7 MBq of radioactive iodine was efficiently captured (> 99%) by gold nanoparticles in various aqueous solutions. Furthermore, the radioactive iodine captured by gold nanoparticles did not leak out. We anticipate that the microbial remediation method developed here will open a new avenue to clean up high radiation contaminated environments. (Grant number: NRF-2017M3D3A1A01037019).

PT112 Metabolic engineering of Cupriavidus necator for isoprene production

Kavita Yadav[1], Marco Garavaglia[1], Naglis Malys[1], Nigel Minton[1]

[1]*University of Nottingham, Synthetic Biology Research Centre, Nottingham, United Kingdom*

Background: *Cupriavidus necator* is a metabolically versatile bacteria with ability to fix CO_2, which makes it an excellent chassis for industrial applications. Isoprene has wide industrial applications such as in synthetic rubber, adhesives and jet fuels. Use of C. *necator* as a microbial cell factory for isoprene production has a great potential. Isoprene is synthesised by isoprene synthase (ispS) from dimethylallyl diphosphate (DMAPP) which is produced by mevalonate (MVA) and methylerythritol phosphate (MEP) pathway.

Objectives: In this study we have engineered *C. necator* for isoprene production by incorporating MVA pathway genes from *Enterococcus faecalis* and *Streptococcus pneumoniae*, and isoprene synthase from *Populus alba.*

Methods: A set of strains were created by transformation and genomic integration containing isoprene biosynthetic pathway. Cultures were grown in serum bottles for isoprene measurment and isoprene quantification was done using gas chromatography.

Results: *C. necator* strain containing ispS under the control of phaC and arabinose promoter produced 4.8 and 6.5 ug/ml isoprene, respectively, in serum bottles containing fructose minimal media. Deletion of by-product, polyhydroxybutyrate (PHB), pathway resulted in 2-fold increase in isoprene production. Genomic integration of isoprene biosynthetic pathway in *C. necator* phaCAB deletion strain resulted in production of 5 ug/ml isoprene. Overexpression of isoprene synthase on plasmid, in addition to a genomic copy of isoprene biosynthetic pathway, resulted in 28.3 and 40.2 ug/ml isoprene production under the control of phaC and arabinose promoter, respectively. Integration of MVA pathway, in addition to native MEP pathway, resulted in up to ten-fold increase in isoprene.

PT113 Systems metabolic engineering of Mannheimia succiniciproducens for succinic acid production based on elementary mode analysis with clustering

Meehee Kim[1], Won Jun Kim[1], Jung Ho Ahn[1], Hyun Uk Kim[1], Sang Yup Lee[1]

[1]*KAIST, Chemical and biomolecular engineering, Daejeon, Korea, Rep. of South*

Background: *Mannheimia succiniciproducens*, a capnophilic gram-negative rumen bacterium, has been employed for the efficient production of succinic acid. Although *M. succiniciproducens* metabolism was previously studied using a genome-scale metabolic model, more metabolic characteristics are to be understood.

Objectives: Elementary mode analysis accompanied with clustering ('EMC' analysis) is used to gain further insights on metabolic characteristics of *M. succiniciproducens* allowing efficient succinic acid production

Methods: Based on the results of EMC analysis, *zwf* gene is identified as a novel overexpression target for the improved succinic acid production. This gene is overexpressed in a previously constructed succinic acid-overproducing *M. succiniciproducens* LPK7 strain. Heterologous *mdh* is later intuitively selected for overexpression to synergistically improve succinic acid production by utilizing abundant NADPH pool mediated by the overexpressed *zwf*.

Results: The LPK7 strains co-expressing mdh alone and both zwf and mdh genes are subjected to fed-batch fermentation to better examine their succinic acid production performances. Strategies of EMC analysis will be useful for further metabolic engineering of *M. succiniciproducens* and other microorganisms to improve production of succinic acid and other chemicals of interest.This work was supported by the C1 Gas Refinery Program funded by the Ministry of Science and ICT through the National Research Foundation of Korea (NRF-2016M3D3A1A01913250).

PT114 Formic acid as a secondary carbon source for succinic acid production by metabolically engineered Mannheimia succiniciproducens

Meehee Kim[1], Jung Ho Ahn[1], Junho Bang[1], Won Jun Kim[1], Sang Yup Lee[1]

[1]KAIST, Chemical and biomolecular engineering, Daejeon, Korea, Rep. of South

Background: Much effort has been exerted to reduce one carbon (C1) gas emission due to the climate change nowadays. As one promising way utilize C1 gas, several technologies have been developed to convert C1 gas into useful chemicals such as formic acid (FA).

Objectives: *Mannheimia succiniciproducens*, a facultative anaerobic gram negative rumen bacteria, was engineered using systems metabolic engineering method to efficiently utilize FA.

Methods: 13C isotope analysis of *M. succiniciproducens* showed that FA could be utilized through formate dehydrogenase (FDH) reaction and/or the reverse reaction of pyruvate formate lyase (PFL). FA assimilation via FDH was found to be more efficient than the reverse reaction of PFL. Four different FDHs from *M. succiniciproducens*, *Methylobacterium extorquens*, and *Candida boidinii* were amplified in the LPK7 strain to find suitable FDH for enhancing FA assimilation.

Results: The result strain produced 76.11 g/L SA with the yield and productivity of 1.28 mol/mol and 4.08 g/L/h, respectively, using sucrose and FA as dual carbon sources. The strategy employed here will be similarly applicable in developing microorganisms to utilize FA and to produce valuable chemicals and materials from FA. This work was supported by the C1 Gas Refinery Program funded by the Ministry of Science and ICT through the National Research Foundation of Korea (NRF-2016M3D3A1A01913250).

PT115 Homo-succinic acid production by systems metabolically engineered Mannheimia succiniciproducens

Meehee Kim[1], Jeong Wook Lee[1], Jongho Yi[1], Tae Yong Kim[1], Sol Choi[1], Jung Ho Ahn[1], Hyohak Song[1], Moon-Hee Lee[1], Sang Yup Lee[1]

[1]KAIST, Chemical and biomolecular engineering, Daejeon, Korea, Rep. of South

Background: Succinic acid (SA) is a four carbon dicarboxylic acid of great industrial interest that can be produced by microbial fermentation. *Mannheimia succiniciproducens*, a facultative anaerobic gram negative rumen bacteria, has been engineered using systems metabolic engineering method to efficiently produce SA.

Objectives: Development of a high-yield homo-SA producing *Mannheimia succiniciproducens* strain by metabolic engineering.

Methods: The PALFK strain (*ldhA*$^-$, *pta*$^-$, *ackA*$^-$, *fruA*$^-$) was developed based on optimization of carbon flux towards SA production while minimizing byproducts formation through the integrated application of in silico genome-scale metabolic flux analysis, omics analyses, and reconstruction of central carbon metabolism.

Results: Fed-batch fermentations of PALFK strain with low- and medium-density (OD_{600} of 0.4 and 9.0, respectively) inocula produced 69.2 and 78.41 g/L of homo-SA with yields of 1.56 and 1.64 mol/mol glucose equivalent and overall volumetric SA productivities of 2.50 and 6.02 g/L/h, respectively, using sucrose and glycerol as dual carbon sources. The SA productivity could be further increased to 38.6 g/L/h by employing a membrane cell recycle bioreactor system. This work was supported by the Technology Development Program to Solve Climate Changes on Systems Metabolic Engineering for Biorefineries (Grants NRF-2012M1A2A2026556 and NRF2012M1A2A2026557) from the Ministry of Science and ICT through the National Research Foundation (NRF) of Korea.

PT116 Development of recombinant Corynebacterium glutamicum overproducing 5-aminovaleric acid

Jae Eun Lee[1], Jae Ho Shin[1;2], Seok Hyun Park[1;2], Young Hoon Oh[3], Jae Woong Choi[1], Moon Hee Lee[1;2], Jae Sung Cho[1;2], Ki Jun Jeong[1], Jeong Chan Joo[3], James Yu[2], Si Jae Park[4], Sang Yup Lee[1;2;5]

[1]KAIST, Chemical and Biomolecular Engineering, Daejeon, Korea, Rep. of South
[2]KAIST, Metabolic Engineering National Research Laboratory and BioProcess Engineering Research Center, Daejeon, Korea, Rep. of South
[3]Korea Research Institute of Chemical Technology, Division of Convergence Chemistry, Center for Bio-based Chemistry, Daejeon, Korea, Rep. of South
[4]Myongji University, Environmental Engineering and Energy, Yongin, Korea, Rep. of South
[5]KAIST, Bioinformatics Research Center, Daejeon, Korea, Rep. of South

Background: 5-Aminovaleric acid (5AVA) has attracted interests as an important five-carbon platform chemical which can be potentially used for synthesis of polymer and other valuable compounds.

Objectives: A recombinant *Escherichia coli* have been developed for producing 5AVA from L-lysine, however attempts to produce 5AVA from simple carbon sources such as glucose resulted in the low yield and productivity. Therefore, we seleted *Corynebacterium glutamicum*, which is capable of producing L-lysine in high level, as a chassis strain and metabolically engineered it for the enhanced production of 5AVA from glucose.

Methods: For optimal expression of *davB* and *davA* from *Pseudomonas putida*, various promoters and origins of replications of expression plasmids were examined. Next, homology modeling was used for finding possible endogenous bottleneck gene.

Results: Among various plasmids, one harboring codon-optimized *davA* fused with poly His-tag and *davB* under a strong synthetic H_{36} promoter performed best for 5AVA production in *C. glutamicum*. The fed-batch fermentation of the selected strain produced 19.7 g/L of 5AVA and 11.9 g/L of glutaric acid. The possible endogenous gene converting 5AVA to glutaric acid was predicted as *gabT* gene by homology modeling, and the *gabT*-mutant strain showed enhanced production of 5AVA up to 33.1 g/L. [This work was supported the Technology Development Program to solve climate changes (Systems Metabolic Engineering for Biorefineries) (NRF-2012M1A2A2026556 and NRF-2012M1A2A2026557) from the Ministry of Science, ICT and Future Planning (MSIP) through the National Research Foundation (NRF) of Korea and NRF grant funded by the MSIP (NRF-2016R1A2B4008707).]

PT117 Development of L-arginine overproducing Corynebacterium glutamicum

Jae Eun Lee[1], Seok Hyun Park[1;2], Hyun Uk Kim[1;3], Tae Yong Kim[1;3], Jun Seok Park[2], Suok-Su Kim[2], Sang Yup Lee[1;3;4]

[1]KAIST, Chemical and Biomolecular Engineering, Daejeon, Korea, Rep. of South
[2]Daesang Corporation Research Center, Incheon, Korea, Rep. of South
[3]KAIST, BioInformatics Research Center, Daejeon, Korea, Rep. of South
[4]KAIST, BioProcess Engineering Research Center, Daejeon, Korea, Rep. of South

Background: L-Arginine is one of important amino acids which has a number of applications in diverse industries including food, health, pharmaceutical and cosmetics. Compared to *Escherichia coli*, *Corynebacterium glutamicum* is advantegeous in producing L-arginine sine it lacks L-arginine degrading enzyme and can utilize broader range of carbon sources.

Objectives: In this study, we developed recombinant *C. glutamicum* strain which can overproduce L-arginine in both lab-scale and industrial-scale bioreactors.

Methods: First, random mutagenesis was performed to find *C. glutamicum* strain more tolerant to L-arginine. For further strain improvement, regulatory repressors of arginine operon were removed, NADPH level was optimized, an exporter of L-glutamate which is a precursor of L-arginine was disrupted, and bottleneck genes involved in the L-arginine biosynthetic pathway were amplified.

Results: Through the random mutagenesis, the strain capable of prducing 2-fold L-arginine was obtained. Two genes (*argR* and *farR*) responsible for negative regulation on L-arginine operon were knocked out, NADPH level increased by enhancing pentose phosphate pathway flux, and the Ncgl1221 gene encoding glutamate exporter was deleted. The following systems metabolic engineering further improved the strain allowed production of 92.5 and 81.2 g/L of L-arginine in 5 L and 1,500 L scale bioreactors, respectively. [This work was supported by the Technology Development Program to Solve Climate Changes on Systems Metabolic Engineering for Biorefineries from the Ministry of Science, ICT and Future Planning (MSIP) through the National Research Foundation (NRF) of Korea (NRF-2012-C1AAA001-2012M1A2A2026556). Further support from Daesang Corporation is appreciated.].

PT118 Production of terephthalic acid by metabolically engineered Escherichia coli

Jae Eun Lee[1], Zi Wei Luo[1], Sang Yup Lee[1;2;3]

[1]*KAIST, Chemical and Biomolecular Engineering, Daejeon, Korea, Rep. of South*
[2]*KAIST, BioProcess Engineering Research Center, Daejeon, Korea, Rep. of South*
[3]*KAIST, BioInformatics Research Center, Daejeon, Korea, Rep. of South*

Background: Terephthalic acid (TPA) is an important commodity chemical which is principally used as a monomer precursor of polyethylene terephthalate. Currently, TPA is chemically synthesized by high energy-requirement and potentially hazardous p-xylene (pX) oxidation process.

Objectives: Compared to the chemical process, the biotransformation process is more advantegous in terms of milder reaction conditions and environmental protection. In order to produce totally bio-based TPA, we developed a recombinant *Escherichia coli* strain which can convert biomass-derived pX to TPA by metabolic engineerig.

Methods: The whole biosynthetic pathway toward TPA from pX was divided into two modules, upstream and downstream, and assembled in different configurations for finding balanced level of gene expressions. After the strain construction, two-phase fermentation was conducted to capture volatile, insoluble and toxic TPA produced by the developed *E. coli* strain.

Results: After validation of upstream and downstream pathways for TPA biosynthesis from pX, expression levels of both modules were varied and the highest TPA production of 145.3 mg/L was obtained in flask culture. In two-phase fed-batch fermentation using oleyl alcohol as carrier phase, the selected strain successfully convert 8.8 g of pX into 13.3 g of TPA. These results suggest the *E. coli* bioconversion system as a promising alternative of the chemical synthesis for TPA production. [This work was supported by the Intelligent Synthetic Biology Center through the Global Frontier Project (2011-0031963) of the Ministry of Science, ICT & Future Planning through the National Research Foundation of Korea.].

PT119 Production of 3-aminopropionic acid by engineering the metabolism of Escherichia coli

Su Jeong Cho[1], Chan Woo Song[1], Je Woong Kim[1], Joungmin Lee[1], Yoo-Sung Ko[1], Sang Yup Lee[1]

[1]Korea Advanced Institute of Science and Technology, Chemical and Biomolecular Engineering, Daejeon, Korea, Rep. of South

Background: 3-Aminopropionoic acid, also called β-alanine, has been proposed to be an intermediate chemical for the production of acrylamide, acrylonitrile, and other nitrogen-containing chemicals. In addition, poly-β-alanine, a polymer of 3-aminopropionic acid has diverse applications, including cosmitics, water purification, and construction. While production of such 3-aminopropionic acid derivatives are currently produced by chemicals reactions in industries, bioproduction of 3-aminopropionic acid can provide a environment-firendly substitutes for the industrial processes.

Objectives: The metabolism of *Escherichia coli* was engineered to produce 3-aminopropionic acid.

Methods: To enable bioproduction of 3-aminopropionic acid in *E. coli*, the *panD* gene coding L-aspartate-α-decarboxylase was overexpressed. To enhance the titer, a fumaric acid-overproducing strain was employed to overexpress the *panD* gene. Finally, the *ppc* gene coding for phoephodenolpyrunvate carboxylase was overexpressed based on 12 different plasmids to identify the optimal strength of the *ppc* gene expression to achive the highest 3-aminopropionic acid titer.

Results: Flask cultivation of the resulting strain produced 3.94 g/L 3-aminopropionic acid, the highest titer reported in flask cultures. Subsequent fed-batch fermentation of the engineered *E. coli* strain produced 32.3 g/L 3-aminopropionic acid in 39 h. [This work was supported by Technology Development Program to Solve Climate Changes on Systems Metabolic Engineering for Biorefineries (NRF-2012M1A2A2026556 and NRF-2012M1A2A2026557) from the Ministry of Science and ICT through the National Research Foundation (NRF) of Korea.]

PT120 Engineering metabolism of Escheichia coli to produce 3-hydroxypropionic acid and malonic acid

Su Jeong Cho[1], Chan Woo Song[1], Je Woong Kim[1], In Jin Cho[1], Sang Yup Lee[1]

[1]Korea Advanced Institute of Science and Technology, Chemical and Biomolecular Engineering, Daejeon, Korea, Rep. of South

Background: 3-Hydroxypropionic acid and molonic acid have been ranked by U.S. Department of Energy as top building block chemicals to be derived from biomass for sustainable production of chemicals through biorefineries. Although engineered *E. coli* strains producing such chemicals using glycerol as a precursor have achived high titers, vitamin B12-dependency and inefficient cofactor regeneration have rendered the strins less competitive. Production of 3-hydroxypropionic acid and malonic acid through β-alanine pathway can overcome such issues and achive better performance of overproducer strains.

Objectives: *Escherichia coli* was metabolically engineered to produce 3-hydroxypropionic aicd and malonic acid through β-alanine pathway.

Methods: The *pa0132* gene from *P. aeruginosa* coding β-alanine pyruvate transaminase was introduced to *E. coli* to convert β-alanine to malonic semialdehyde. To produce 3-hydroxypropionic acid from malonic semialdehyde, the *ydfG* gene from *E. coli* coding for molonic semialdehyde reductase was overexpressed and the native promoter of the *sdhC* gene was replaced with the *trc* promoter. To produce malonic acid from molonic semialdehyde, the *yneI* gene form *E. coli* coding for semialdehyde dehydrogenase was overexpressed and the *ydfG* gene was deleted.

Results: Flask cultivation of the resulting strains produced 3.69 g/L 3-hydroxypropionic acid and 0.450 g/L malonic acid, respectively. Subsequent fed-batch fermenation of the engineered strains produced 31.1 g/L 3-hydroxypropionic acid and 3.60 g/L malonic acid, respectivey. [This work was supported by Technology Development Program to Solve Climate Changes on Systems Metabolic Engineering for Biorefineries (NRF-2012M1A2A2026556 and NRF-2012M1A2A2026557) from the Ministry of Science and ICT through the National Research Foundation (NRF) of Korea.]

PT121 Efficient self-assembled microRNA delivery system consisting of PEGylated ploycationic polymer and cholesterol-conjugated microRNA for tumor treatment

Byeonggeol Mun[1], Eunji Jang[2], Seungmin Han[3], Hye Young Son[4], Yuna Chol[4], Yong-Min Huh[4], Seungjoo Haam[1]

[1]Yonsei University, Chemical and Biomolecular Engineering, Seoul, Korea, Rep. of South
[2]Novomics Co. Ltd, MediBio-Informatics Research Center, Seoul, Korea, Rep. of South
[3]The University of Arizona, Department of Surgery, Tucson, United States
[4]Yonsei University, Radiology, Seoul, Korea, Rep. of South

Background: MicroRNA (miR), a key molecule involved in endogenous RNA interference (RNAi), is a promising therapeutic agent. *In vivo* delivery of miR, however, is a major factor limiting its application because its polyanionic nature and vulnerability to breakdown make delivery of miR to targeted lesions difficult.

Objectives: To overcome these challenges, we developed a self-assembled miR delivery system consisting of cholesterol-conjugated miR and polyethylene glycol-grafted polyethylene imine.

Methods: Nanosized complexes of miR with polyethylene imine (PEI), which protected miR and its delivery into targeted lesions *in vivo*, were successfully synthesized by polyethylene glycol (PEG) grafting. The hydrophobicity of cholesterol improved the structural stability of the complex, preventing the loss of miR.

Results: Here, we report the preparation of this self-assembled complex. We examined the delivery efficiency and validated the therapeutic efficacy of the complex. In conclusion, our miR delivery system proposed considerable potential for effective *in vivo* delivery of miR.

PT122 Fluorescent dye conjugated arbidol based on in silico design for rapid detection of arbidol-resistant influenza virus

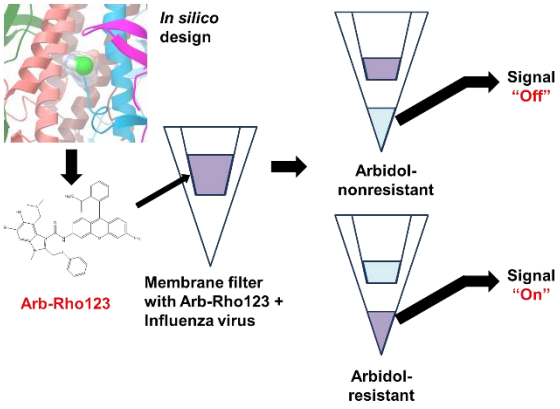
Jinyoung Kim[1], Seungjoo Haam[1], Daesub Song[2], Hyun-Ouk Kim[2], Aram Kang[2]

[1]*Yonsei University, Chemical & Biomolecular Engineering, Seoul, Korea, Rep. of South*
[2]*Korea University Sejong Campus, College of Pharmacy, Sejong, Korea, Rep. of South*

Background: Arbidol, a promising anti-influenza agent, is considered as a good substitute of Oseltamivir (Tamiflu). As the rate of Oseltamivir-resistant influenza virus has been increasing, Arbidol's distinct mechanism that inhibits viral membrane fusion could be applied for that. Meanwhile, viral resistance against Arbidol needs to be studied further, therefore the necessity of rapid detection of it has been risen.

Objectives: Rapid detection of Arbidol-resistant influenza virus by fluorescent dye conjugated Arbidol which is designed based on *in silico* docking simulation.

Methods:



First, In silico design of fluorescent dye conjugated Arbidol, which fits on the Arbidol binding pocket in Influenza virus Hemagglutinin. Then, synthesize Arbidol-dye via EDC-NHS mechanism based on previous simluation. Subsquently, react synthesized Arbidol-dye with Influenza virus. Finally, filter centrifuge and detect its fluorescent intensity.

Results: In silico evaluation of the various Arbidol-fluorescent dye demonstrates that Arb-Rho123 could be an ideal agent for the system. Arb-Rho123 is successfully synthesized via EDC-NHS coupling reaction. The fluorescence intensity after centrifuging with membrate filter implies that the Arb-Rho123 is adsorbed on the docking site of Hemagglutinin.

PT123 A potential diagnosis motif of disease based on structural property of inverse opal hydrogels

Hyo Lee[1], Moo-kwang Shin[1], Woo-seok Jhin[1], Swungjoo Haam[1]

[1]Yonsei University, Chemical engineering, Seoul, Korea, Rep. of South

Background: Inverse opal hydrogels (IOHs) has regularly arranged opal-shape void spaces. The porous structure enhances permeability and deformability of the hydrogels. IOH also has photonic property based on the size of the pores and their arrangement. External stimulus can lead volumetric change of pores and diffraction wavelengths shift. With this feature, IOHs are widely studied for target responsive sensors

Objectives: In particular, with simple alteration in the components of hydrogels, IOHs can be presented as biosensor. The humoral pH-ionic concentration, and specific proteins are representative markers in vital reactions or immune responses. It's important to following up this parameters for early diagnosis of disease. We designed the multi-responsive hydrogel sensors to detect aforementioned targets and tested performance of the sensor.

Methods: Herein, we fabricated high quality biomarker responsive IOHs film by templating photo-polymerization of hydrogel monomers on a three-dimensional (3D) silica nanoparticles (SNPs) colloidal array. First, the array was prepared by self-assembly of monodisperse SNPs. Next, we backfilled the array with modified hydrogel precursor materials, followed by cross-linking using UV light. After removal of the SNPs array with hydrofluoric acid, we finally obtained macroporous IOHs.

Results: To summarize the results, we monitored the diffraction peak shift with controlling the pH and glucose concentration. The IOHs film responded to biomarkers through volume change with increased donnan potential through the hydrogels. With the protein detection we introduced FRET dye modified antibody pair to the IOHs and confirmed the binding of antigen to antibodies which are conjugated to IOH backbones.

PT124 The Discrete Acyltransferase KirCII as Potential Tool for Synthetic Biology of Polyketide Compounds

Ewa Maria Musiol-Kroll[1], Florian Zubeil[2], Stephanie Grond[2], Gavin Williams[3], Sang Yup Lee[4], Tilmann Weber[4], Wolfgang Wohlleben[1]

[1]Eberhard Karls University of Tübingen, Microbiology/Biotechnology, Tübingen, Germany
[2]Eberhard Karls University of Tübingen, Institut für Organische Chemie, Tübingen, Germany
[3]North Carolina State University, Department of Chemistry, Raleigh (NC), United States
[4]Technical University of Denmark, The Novo Nordisk Foundation Center for Biosustainability, Kgs. Lyngby, Denmark

Background: The biosynthesis of the polyketide antibiotic kirromycin requires the activity of two discrete acyltransferases (ATs), KirCI and KirCII. KirCI is a malonyl-CoA specific AT and loads malonate onto the assembly line. The discrete AT KirCII was confirmed to be specific for ethylmalonyl-Coenzyme A (ethylmalonyl-CoA) and only one acyl carrier protein (KirACP5). More recently, *in vitro* studies revealed that KirCII can also use other nonmalonyl-CoA substrates such as allylmalonyl-CoA, propargylmalonyl-CoA, and to a lesser extent azidoethyl and phenylmalonyl-CoA for KirACP5-loading.

Objectives: The fact that KirCII continued to be the only characterized discrete AT with such a promising spectrum of substrate specificity, encouraged us to study the flexibility of KirCII in *in vivo* experiments.

Methods: In our "bioderivatization" approach, the malonyl-CoA synthetase T207G/M306I MatB was introduced into the kirromycin producer strain. Non-natural precursors, allyl- and propargylmalonic acid, were fed to the modified strain. The engineered strain activated the substrates to CoA-forms, which were subsequently utilized by KirCII for the polyketide biosynthesis.

Results: The feeding experiments resulted in the production of the allyl- and propargyl-kirromycin. The latter was utilized as educt for further derivatization by "click" chemistry, which led to the generation of a fluorescent kirromycin product, coumarin-kirromycin. The study demonstrates the promiscuity of the MatB-KirCII/ACP5 system, which can be exploited for the development of a molecular tool for generation of polyketide derivatives. The combination of this "bioderivatization" approach and "click" chemistry methods may enable the production of novel molecular probes and analogues of compounds with valuable bioactivities.

PT125 Bacterial whole cell biosensors for the remote detection of buried explosive devices

Etai Shpigel[1], Benjamin Shemer[1], Tal Elad[1], Anat Glozman[1], Shimshon Belkin[1]

[1]*The Department of Plant & Environmental Sciences, The Institute of Life Sciences, The Hebrew University of Jerusalem, Israel*

Background: Landmines and explosive remnants of war pose a global problem which claims many lives and casualties long after the conflict has ended. Current approaches for the location of landmines, such as metal detection, require physical presence at the minefield and therefore involve high risk to personnel; these methods are also costly, time consuming, and have a high rate of false positive results. The development of alternative technologies for efficient remote detection of buried explosive devices is thus urgently needed.

Most landmines contain 2,4,6-trinitrotoluene (TNT) as the main explosive component, as well as its production byproduct 2,4-dinitrotoluene (DNT). Over time, the more volatile DNT leaks out of the landmine casing and accumulates in the soil above it, serving as an excellent "signature molecule" for its presence.

Objectives: Development of bacterial whole cell biosensors for the remote detection of buried explosive devices.

Methods: Over the last few years we have developed *Escherichia coli*-based microbial bioreporters for landmine detection. These sensor strains harbor fusions of gene promoters (*yqjF* and *azoR*) induced by the presence of DNT metabolites to the bioluminescence *luxCDABE* reporter gene cassette.

Results: Several molecular biology and encapsulation techniques were applied to improve the sensitivity of these bioreporters, resulting in a ca. 100-fold reduction in the DNT detection threshold, as well as in a significant expansion of the temperature operation range. We have demonstrated the feasibility of using drones for airborne imaging of the bioluminescent signal emitted by the bacteria, and detected real anti-personnel landmines buried in a sandy soil.

PT126 Enhancement of 2,3-Butanediol Production in Escherichia coli Using Response Regulator DR1558 Derived from Deinococcus radiodurans

SeongJu Park[1], Jong-il Choi[1]

[1]Chonnam National University, Biotechnology & Bioengineering, Interdisciplinary Program for Bioenergy & Biomaterials, Gwangju, Korea, Rep. of South

Background: We used DR1558 gene from *D. radiodurans*, known as Response Regulator gene. There is a report on the productivity increase through introduction of DR1558 gene in succinic acid production. Likewise, productivity of 2,3-BDO is expected to increase through introduction of DR1558 gene.

Objectives: We inserted DR1558 gene from *D. radiodurans* into a strain capable of producing 2,3-BDO in order to tolerate external environmental stress. The insertion of DR1558 gene into E. coli is aimed at increasing 2,3-BDO production. In addition, we investigated the effect of DR1558 gene on genes related to the metabolic pathway of *E. coli*.

Methods: All of the transformed *E. coli* strains (DH5a pKM212_ydjlalssd, DH5a pKM212_ydjlalssd pRadGro and DH5a pKM212_ydjlalssd pRadGro_DR1558) were all cultured with LB medium supplemented with the required antibiotics. The yield of byproducts was measured using HPLC and the expression levels of genes related to metabolic pathways in the exponential phase and the stationary phase were analyzed using qRT-PCR.

Results: We produced 2,3-butanediol from *E. coli* using alsS, alsD, and bdhA genes from *B. subtilis*. When *E. coli* DH5a pKM212_ydjlalssd strain was cultured at 37 ° C in the initial experiment, 3.18 g / L 2,3-BDO was produced. Then, pRadGro vector and pRadGro_DR1558 vector were transformed and cultured at 37 ° C, respectively. The concentration of 2,3-BDO (control: 3.18 g / L, empty vector: 1.97 g / L, pRadGro_DR1558: 7.99 g / L) was found to be higher in the strain containing DR1558 gene at 37 °C.

PT127 Targeting sex hormones and analogue compounds using recombinant yeast-based bioreporters coupled with high-performance thin layer chromatography.

Liat Moscovici[1], Nidaa Abu-Rmailah[1], Carolin Riegraf[2], Dror Shkibai[1], Hadas Atias[1], Sebastian Buchinger[2], Georg Reifferscheid[2], Shimshon Belkin[1]

[1]*The Hebrew University of Jerusalem, Giv'at Ram, Jerusalem, Israel*
[2]*Federal Institute of Hydrology, Koblenz, Germany*

Background: The persistence of endocrine disrupting compounds (EDCs) throughout wastewater treatment processes pose a significant threat to ecosystems and human health. These chemical agents bind to human hormone receptors and either activate or inhibit natural endocrine system functions, leading to diverse potentially adverse effects.

Objectives: Development of methodology for the multidimensional detection of EDCs in the environment.

Methods: The yeast *Saccharomyces cerevisiae* is an ideal model system for detecting steroid hormones and analogues, as it lacks endogenous steroid receptors. We designed a battery of fluorescent yeast strains expressing either the human estrogen receptor alpha (ERα) or androgen receptor (AR). Upon binding the ERα- or AR-ligand complexes to the specific hormone response elements, each strain expresses green, red or blue fluorescent proteins. These bioreporters are used to identify and quantify the presence of endocrine disrupting compounds in environmental samples. Coupling of this bioassay to thin layer chromatography, a standard method for EDCs separation, a wide variety of EDCs can be screened simultaneously.

Results: The combination of chemical separation with an optical bioassay is a valuable complementary approch to available chemical detection methods, enabling the detection of compounds with hormonal-related bioactivity, even when present in complex samples, thus alleviating the need for chemical analysis of the entire sample. The combination of different reporter proteins allows the detection of several EDC groups in a single assay, thus improving the robustness and cost-effectiveness of the procedure.

PT128 Monitoring of Synthetic Biology: Recent Developments and Possible Implications on Biosafety

Stefanie Siche[1], Swantje Straßheim[1], Anke Stein[1;2]

[1]Federal Office of Consumer Protection and Food Safety (BVL), Division 402 - Secretariat to the Central Committee on Biological Safety (ZKBS), Berlin, Germany
[2]Head of Secretariat to the ZKBS

Background: Synthetic Biology is a fast evolving field using engineering principles to design new biological systems and living entities for novel applications. Researchers aim to produce novel pharmaceuticals, vaccines, or food additives. The progress is enormous. However, there is no clear definition of Synthetic Biology and discussions on potential hazards and the need for dedicated regulations are ongoing.

In Germany, the Central Committee on Biological Safety (ZKBS), an independent expert panel instituted by the German Federal Government and responsible for evaluating genetically modified organisms (GMOs) with regard to their potential risks for humans, animals and the environment, attentively monitors new developments in Synthetic Biology and regularly reports to the public.

Objectives: Here, we summarize the milestones achieved in the five major fields of Synthetic Biology along with biosafety considerations of the ZKBS.

Results: Currently, the lines of research in Synthetic Biology do not entail new risks for biosafety compared to classical genetic engineering. As most of the approaches generate GMOs, their potential risk can be assessed using existing methodologies as outlined in the European Directives 2001/18/EC and 2009/41/EC. However, replicating protocells might be generated at some time in the future and might necessitate additional regulation as they do not have a natural counterpart as a basis for risk assessment

Both researchers as well as risk assessors should closely follow the developments in the dynamic field of Synthetic Biology in order to identify regulatory gaps and to establish an appropriate risk assessment.

PT129 Enhancing Xylitol Production of Candida guilliermondii by Genetic Engineering

Denise Atzmüller[1], Nadine Ullmann[1], Alexander Zwirzitz[1]

[1]*University of Applied Sciences Upper Austria, Wels, Austria*

Background: Xylitol is a sugar-alcohol, which is of great interest to the food industry. Due to its comparable sweetening power with 40 % less calories than sucrose and its insulin independent metabolism, it is a suitable ingredient for different types of diabetic foods. *Candida guilliermondii*, a non-conventional yeast, is known for its ability to produce xylitol naturally and could therefore offer a biotechnological alternative to the current chemical xylitol production.

Objectives: So far, no studies describing the use of molecular tools to enhance the production of xylitol in *C. guilliermondii* are published. Therefore, we focused on the generation of a genetically engineered *C. guilliermondii* strain with improved xylitol production.

Methods: We identified two enzymes involved in the xylose metabolic pathway that play a major role in xylitol production: xylose dehydrogenase (XDH1) and xylose reductase (XYL1). By molecular cloning, we generated an XDH1 knockout strain, an XYL1 overexpressing strain and a strain that combined both modifications. We investigated the respective enzyme activities and finally, xylitol yield in batch fermentations.

Results: Not surprisingly, the XDH1 knockout and combined XDH1 knockout/XYL1 overexpressing strain showed deficiencies when grown on xylose as a sole carbon source. However, by using glucose as a co-substrate, growth can be restored up to 78 %. Further, we could demonstrate the successful alterations in the substrate converting efficiency of XYL1-overexpression and XDH1 knockout strains. Finally, the effect of the single and combined genetic modifications on xylitol yield were investigated and showed a significant enhancement, especially if both alterations were combined.

PT130 The Antiviral Activity of Streptomyces spp. Against Orchid Odontoglossum Ringspot Virus (ORSV)

Kuan-Chih Tsai[1], Bing-Lan Liu[1], Meng-Jen Lee[1], Chin-An Chang[1]

[1]*Chaoyang University of Technology, Applied Chemistry, Taichung, Taiwan*

Background: Virus infections has been important limiting factor in orchids cultivation because it could decrease the orchids quality. Odontoglossum ringspot virus (ORSV) is one of the most reported viruses infecting orchids, which spread widely in the world.

Objectives: To evaluated the effectiveness biocontrol agent against ORSV.

Methods: Samples collection of this study were conducted in several locations of orchids cultivation areas in Taiwan. The organisms were screening througt the indirect ELISA assay to confirm the antiviral activity of the cultivation filtrate.

Results: The actinomycete was isolated from sediment soil and identified as *Streptomyces* based on the colony morphology, named CA5 and SML1. Indirect ELISA assay was employed to confirm the antiviral activity of the target protein against ORSV infected orchid plant. The optium antiviral activties were observed while cultivated in 0.5% soybean milk medium for both strains. The best antiviral activity was observed at 7^{th} and 28^{th} days after cultivation for CA5 and SML1, respecyively. The active fragment was observed at the band appeared around ~25 kDa in the SDS-PAGE. This band is excised from the gel and subjected to in-gel trypsin digestion and analyzed by MALTI-TOF Mass Spectrometry. Furthermore, the culture filtrate of *Streptomyces* was precipitated by 60% $(NH_4)_2SO_4$ and further purified by DEAE ion-exchange chromatography followed by Sephacryl S-200 gefiltration chromatography. In conclusion, the isolated *Streptomyces* culture has shown to be effective in the eradication of ORSV from infected orchid, and thus has the potential to be a good biocontrol agent for eliminate the virus contaminated orchid.

PT131 Prediction of novel non-coding RNAs relevant to the adaptation of Pseudomonas putida grown in a bioreactor

Gil Graça-Lopes[1], Vânia Pobre[1], Margarida Saramago[1], Patrícia Apura[1], Andreas Ankenbauer[2], Ralf Takors[2], Cecília M. Arraiano[1], Sandra Viegas[1]

[1]ITQB NOVA, Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Portugal, Oeiras, Portugal
[2]Institute of Biochemical Engineering, University of Stuttgart, Stuttgart, Germany

***** The authors Gil-Graça Lopes and Vânia Pobre should be considered as equal contributors to this work

Background: *Pseudomonas putida* is a microorganism with great potential for industry. Easy manipulation of its metabolic pathways to produce complex compounds makes *P. putida* an extremely useful bacterial factory, but further optimization is required to improve production yields. An efficient way to control bacterial metabolism is through the manipulation of its regulatory RNAs, molecules capable of controlling the levels of both mRNAs and proteins in the cell. However, knowledge of non-coding RNAs (ncRNAs) in *P. putida* is still quite limited.

Objectives: Prediction of potentially important ncRNAs to the adaptation of *Pseudomonas putida* to bioreactor conditions.

Methods: *P. putida* was grown continuously in a bioreactor and samples were collected at twelve distinct time points. RNA-Seq of the samples was performed and the resulting data was used to predict putative ncRNAs. For this purpose, we relied on sRNA-Detect, a program specifically designed for the prediction of bacterial ncRNAs and on the genome browser Artemis to detect novel features from regions with high expression levels. We obtained 1303 potential ncRNAs with sRNA-Detect and 1001 with Artemis. Both results were compared and only the regions identified by both approaches were further analysed.

Results: We were able to predict 725 novel ncRNAs, of which 335 candidates were in the sense strand and 390 were in the antisense strand. We also found varying levels of expression of these ncRNAs under specific stress condition in the cultivation.

PT132 scfv constrution anti-lt toxin for etec detection

Camila Henrique Pinto[1], Daniela Luz[1], Ana Carolina Dos Santos Leite[1], Roxane Maria Fontes Piazza[1]

[1]*Insituto Butantan, Bacteriologia, São Paulo, Brazil*

Background: Enterotoxigenic *Escherichia coli* (ETEC) is the main pathogen involved in cases of watery diarrhea, especially in children under five years of age, as well as in travelers' diarrhea. One of the toxins produced by this pathotype is the heat-labile (LT) enterotoxin, which can be detected by immunodiagnostic assays, though they are appropriate, polyclonal and monoclonal antibodies are required. As alternative, single chain fragment variable (scFv) can be used for immunodiagnosis, since their production can be faster and at low cost.

Objectives: The anti-LT toxin scFv construction driving its use as tools for ETEC immunodiagnosis.

Methods: The ideal structure for scFv LT was the variable light domain (VL) bound to the heavy variable domain (VH) by a binding peptide, to which specific primers were designed and acquired. The conditions for amplification of the two domains by the PCR technique were standardized; annealing temperatures of 50, 55 and 60ºC were evaluated. The observed amplifications were purified and submitted to Sanger sequencing. For the junction of the two domains, the reactions were compared the use of Platinum Taq DNA Polymerase High Fidelity and recombinant DNA Polymerase plus 0.75 and 1.5% DMSO.

Results: The results showed that the PCR product obtained at 55 °C corresponds to the VL and VH domains of the scFv-LT, both domains were amplified together in all conditions, also the recombinant Taq DNA Polymerase showed amplifications in all analyzed conditions. Therefore, the achievement of scFv-LT toxin turns even nearer for ETEC detection.

PT133 Sustainable management of keratin waste using synthetic fused keratinolytic peptidase SynKer-TT

Audrius Gegeckas[1], Renata Gudiukaite[1], Jonas Virkutis[1], Egle Lastauskiene[1]

[1]*Institute of Biosciences, Microbiology and Biotechnology, Vilnius, Lithuania*

Background: Solid keratin-rich waste management is one of essential research area in nowadays. Conventional chemical and high thermal keratin waste decomposition methods are fully explored and not enough effective for future biotechnology perspective. However, traditional keratin-rich waste decomposition methods could be replaced by environmentally-friendly and economical microbial keratin waste biodegradation methods without energy wastage and essential amino acids and nutrition elements loss. Therefore, microbial bio-decomposition is attractive approach to keratin or keratin-like waste manage without any nutrition loss in eco-friendly process environment.

Objectives: Effectively alter functionality of keratinolytic peptidase by protein engineering using fusion protein technology.

Methods: Bioinformatic methods were used for analysing keratinolytic proteinase GEOker gene. SynKer-TT was constructed by protein engineering joining two protein domains by a protein linker GGSEL. Construct was cloned into pET-28c(+) vector and expressed in *Escherichia coli* BL21(DE3). SynKer-TT was purified by affinity chromatography using immobilized nickel ions and activity was determined using keratin azure as substrate. Obtained hydrolysis products by artificial protein and value of small bioactive peptides were investigated by thin-layer chromatography and compared with commercial peptides generated by non-biological physical treatment and peptides obtained from enzymatic biodegradation by natural GEOker.

Results: The successful construction of a recombinant fusion proteins requires two indispensable parts – the component protein and the linker. The selection of a suitable linker to join the proteins together can be complicated. The reduced activity of SynKer-TT was only one unfavourable subsequence. However, our results showed that thermal stability can be improved by joining two identical protein domains.

PT134 Effect of microbial consortium on destruction of agricultural plant residues

Raimonda Mažylytė[1], Justina Smeledyte[2], Jonas Virkutis[3], Audrius Gegeckas[3]

[1]*Faculty of Natural Sciences, Kaunas, Lithuania*
[2]*Agriculture Academy, Kaunas, Lithuania*
[3]*Institute of Biosciences, Microbiology and Biotechnology, Vilnius, Lithuania*

Background: Soil organic matter is composed of living plant and microbial biomass, dead roots and other plant residues in various stages of decay and soil humus. It is assumed that soil humus forms as a result of microbial activity but little is known about the exact mechanisms of humus formation. Cellulose and lignin are major sources of plant biomass. Each polymer is degraded by a variety of microorganisms which produce enzymes that work synergistically. The intensity of mineralization of plant residues directly depends on soil properties and activity of microorganisms. Inactivation of vegetal residue mineralization results in the accumulation of lignin and phenols in the soil. However, today's microbiological products can successfully accelerate the mineralization of plant residues and contribute to the increase of humus reserves.

Objectives: To determine the effect of microbial preparation RUINEX on plant residues – winter wheat, winter rapeseed and spring rapeseed.

Methods: All microorganisms were isolated and identified by molecular analysis. Lab-scale 5 L bioreactor was used for analysis of growth parameters. After batch fermentation cells were separated by centrifugation. Enzymatic activities in supernatant were analysed and used for treatment of various plant residues to alter their structure. Efficiency of plant residues hydrolysis was analysed by dual column tabletop.

Results: The research of this experiment says that the *Bacillus* spp. bacteria affect the mechanical breaking and cutting characteristics of winter wheat, winter and spring rapeseed residue. The energy which needed to cut and break the residues with microbiological fertilizer is less than without the treatment.

PT135 Development of the Swedish National Research Programme on Antibiotic Resistance

Patriq Fagerstedt[1], Jan-Ingvar Jönsson[2]

[1]*Vetenskapsrådet/Swedish Research Council, Department of Research Policy, Stockholm, Sweden*
[2]*Vetenskapsrådet/Swedish Research Council, Secretary General Medicine and Health, Stockholm, Sweden*

Background: The Swedish Research Council was commissioned by the Swedish Government to initiate a challenge-driven 10-year National Research Programme on Antibiotic Resistance (ABR) as part of the Research and Innovation Bill in 2017.

Objectives: The programme aims to plan, coordinate, and fund ABR research based on strategic priorities and societal needs. The programme should create synergies among funders, prevent overlap and fragmentation by supporting joint activities, efficient use and sharing of data and infrastructures, and provide links to international research initiatives. It should facilitate cross-sectoral and multi-disciplinary research to address identified knowledge gaps and increase the impact of research in society through innovation, capacity building, and evidence-based policy, interventions, and healthcare measures to reduce the burden of ABR.

Methods: The Swedish Research Council together with 10 other national agencies and institutes has set up a Programme Committee to develop a vision, a strategic research agenda with a plan for implementation based on the Swedish strategy to combat antibiotic resistance and feedback from researchers and stakeholders, including representatives from industry, health care, agriculture and patient organisations.

Results: The process and procedures to develop the programme is described including a mapping and analysis of national research funding for ABR, research output in terms of publications and international collaborations, and patents. Relationships of policy level strategies and programme implementation (call formulation, evaluation and selection procedures are explored. Interaction and alignment between objective-driven national programmes and the next ”mission”-oriented European Framework Programme will be discussed.

PT136 Interdisciplinary approach and use of active learning methods Master Program of Applied Microbiology at university

Karen Trchounian[1], Armen Trchounian[1]

[1]*Yerevan State University, Biochemistry, Microbiology and Biotechnology, Yerevan, Armenia*

Background: Nowadays Master programs in Microbiology and related fields is of interest as industry needs more qualified specialists in this area. Current trends are to combine interdisciplinary subjects with professional ones for training students according to the requirements of the labor market.

Objectives: Main goal of the work was to show the effectiveness of introducing new subjects and applied methods in Master program in Applied Micobiology which contains interdisciplinary approach.

Methods: To achieve the goal and to train qualified students via ”Active Learning” methods (online learning tools such as menti, flipped classroom etc.) are applied to have more engagement of students not only in lecture but also lab courses.

Results: At Yerevan State University Microbial Biotechnologies and Biofuel Innovation Center Applied Microbiology Master program has been introduced with many interdisciplinary subjects among them “Innovative technologies in production”, “Innovation, Business modeling, “start-up”, “From Science to Production”, “Chromatographic methods”, “Alternative and Renewable Energy and Energy Security” etc. These approaches strengthen the student’s qualifications and educational outcomes will be satisfactory. Moreover, employing “Active Learning” methods such as “Scavenger Hunt”, “Flipped Classroom”, use of online tools like “Menti” etc. enhances the student’s engagement in the classroom. Taken together it can be concluded that introducing such interdisciplinary subjects and applying active learning methodologies results in 80% increase of student’s qualifications of proposed educational outcomes and the feedback of students about the approaches and the results is nearly to 100% satisfactory.

PT137 History of Microbiology - The Van Musschenbroek Microscope

Lesley Robertson[1], Koen Quint[2]

[1]*Delft University of Technology, Science Centre, Delft, Netherlands*
[2]*Leiden University Medical Centre, Department of Dermatology, Leiden, Netherlands*

Background: Historical microbiology involves repeating old experiments to test the methods and equipment. Experiments using exact replicas of Van Leeuwenhoek microscopes have previously shown what they could do. Good replicas are useful because they are less fragile and less valuable than originals. However, it must be remembered that the quality of replica lenses is not necessarily comparable with the lenses of new (at the time) or centuries-old originals. Observations with Van Leeuwenhoek replicas have given good insight into the possibilities of single lens microscopes at a time when many considered them better than available compound microscopes, but comparison with equivalent microscopes made by other microscopists is necessary.

Objectives: This poster reports investigations into how an original high power Van Musschenbroek microscope (provided by the Dutch Historic Microbiology Foundation) must have been used, and shows the results obtained.

Methods: The microscope was tested using several lenses and samples from yeast to insect wings on small cover-slips.

Results: The most interesting result came from the mode of use. The screws previously claimed to be a sophisticated focusing system actually hold the sample in place and focus is achieved with 2 hinges at the base of the lens holder. Photographs taken through the microscope were good, but a little hazed, possibly because the lenses are over 300 years old. As with the Van Leeuwenhoek microscopes, the view through the microscope with the naked eye was always better than that obtained photographically.

PT138 The JPIAMR Virtual Research Institute

Laura Plant[1]

[1]*Vetenskapsrådet, Research Policy, Stockholm, Sweden*

Background: JPIAMR is a global collaborative organisation engaging 27 nations to curb antibiotic resistance (AMR) with a One Health approach. To date JPIAMR has supported research projects and networks, with funding of approximately 67 million Euro, within the six priority areas of the shared JPIAMR Strategic Research Agenda – therapeutics, diagnostics, surveillance, transmission, environment and interventions.

Objectives: JPIAMR recognises a need to reinforce alignment of research and to address this need the JPIAMR is in the process of establishing the JPIAMR Virtual Research Institute (JPIAMR-VRI), a Global Network connecting research performing organisations including institutes, centres, and infrastructures to each other across sectorial and geographic boundaries in a larger global network.

Methods: The JPIAMR-VRI will provide a platform to increase coordination, improve visibility of the AMR researcher base and facilitate knowledge exchange and capacity development across the globe, covering the full One Health spectrum. Eight networks were awarded funding within the JPIAMR 2018 call for transnational networks “Building the Foundation of the JPIAMR Virtual Research Institute.”

Results: During 2019, these networks will investigate research community needs and develop practical solutions to aid JPIAMR in the development of the JPIAMR-VRI.

PT139 Implementation of MicroMundo@UPorto: a Pedagogical Project of Service-Learning for Education in Microbiology and Antimicrobial Resistance Awareness

Patricia Antunes[1;2], Carla Novais[2;3], Ângela Novais[2], Filipa Grosso[2], Teresa Goncalves Ribeiro[2], Joana Vanessa Cordeiro Melro Mourão[2], Svetlana Ugarcina Perovic[2], Joana Rocha[2], Andreia Rebelo[2], Magdalena Ksiezarek[2], Ana Raquel Freitas[2], Luisa Maria Vieira Peixe[2;3]

[1]*Faculty of Nutrition and Food Science, University of Porto, Porto, Portugal*
[2]*UCIBIO/REQUIMTE, University of Porto., Porto, Portugal*
[3]*Faculty of Pharmacy, University of Porto., Laboratory of Microbiology, Porto, Portugal*

Background: Small World Initiative (originally from the USA) is a successful experimental educational project based on Service-Learning implemented in Spain since 2016/2017, which intends to contribute for solving the societal challenge of Antimicrobial Resistance (AMR) across clinical-food-environmental areas ("One Health").

Objectives: To adapt and implement this project in Portugal under MicroMundo@UPorto designation, within two Curricular Units-UCs (Bacteriology-Pharmacy Faculty, and Microbiology-Nutrition and Food Science Faculty) of Porto University, and to estimate its impact in the improvement of university students' academic performance, acquisition of social/soft skills and AMR awareness.

Methods: University-students, coached by university professors/researchers, organize 4 weekly sessions (2h/each) and teach Basic/Secondary school-students, aiming to discover microorganisms producing new antibiotics while exploring microbial diversity of Portuguese soils. After MicroMundo@UPorto announcement, 45 university-students volunteered to participate in 8 teams (5-6 university-students+1-2 supervisors), and to be responsible for one class (20-25 school-students) from one of the three involved Porto schools. After theoretical and laboratory classes, students are expected to work as a team and meet with their supervisor for schools sessions' preparation (S1-S4). At the end of the project, survey-based evaluations will be applied to university-students.

Results: School sessions (February-March 2019) comprise: S1-concepts (AMR+ biodiversity) and experimental design, including for soil collection (total-n≅80); S2-soils processing; S3-antibiosis assays on selected colonies (total-n≅1600); S4-results interpretation and discussion. Besides AMR awareness, we expect to improve university-students' perception on the effects of Microbiology-UCs on professional practice and an enrichment in autonomy, responsibility/commitment, planning, public communication, teamwork, improvisation and empathy, essential skills for better prepared future health professionals.

PT140 Questionnaire-based exercise based on population awareness and risk perception of antimicrobial resistance

Elena-Alexandra Oniciuc (Alexa)[1], Eleni Likotrafiti[2], Alberto Garre[3], Lorena Ruiz[4], Miguel Prieto[1], Avelino Alvarez-Ordóñez[1]

[1]*Universidad de León, León, Spain*
[2]*Alexander Technological Educational Institute of Thessaloniki, Sindos, Greece*
[3]*Rectory Polytechnic University of Cartagena, Cartagena, Spain*
[4]*Instituto de Productos Lácteos de Asturias - CSIC, Villaviciosa, Spain*

Background: To tackle the antimicrobial resistance (AMR) crisis is of outmost importance for the general population to understand the severity of this phenomena and the relevance of different routes of transmission that could impact on their health.

Objectives: To gather information on the population knowledge, awareness and opinion on different topics related to antibiotics use and AMR, which will help develop new strategies and policy agenda at European level increasing awareness in antimicrobials use amongst consumers.

Methods: Consumers of different age groups, educational and occupational backgrounds, areas of living, diet and household composition participated in an online survey. The questionnaire included questions related to socio-demographic characteristics, personal use of antibiotics, awareness, general knowledge, sources of information, behavior and attitude towards antibiotics, and risk perception on antibiotics and AMR. Descriptive and logistic regression analyses were carried out using different statistical software.

Results: A total of 1,252 respondents, mainly from EU, completed the survey. 57.7% declared they consumed antibiotics in the last year, and some misguided behaviors, especially for those not having a food- or health-related background, were identified. Indeed, respondents from this group more frequently failed in giving the right answer to a series of incontrovertible true/false questions (ANOVA, $p < 0.05$). The youngest respondents were less confident on the information received from traditional mass media (OR=0.425), the national government (OR=0.462), and consumer organizations (OR=0.497), while they frequently obtained information from social networks and online media, which could therefore be exploited as a channel for educational campaigns targeting this population group.

PT141 Historical microbiology - Antoni van Leeuwenhoek's experiments with insect corneas.

Lesley Robertson[1]

[1]*Delft University of Technology, Science Centre, Delft, Netherlands*

Background: Antoni van Leeuwenhoek has been called secretive about his methods, but this was not always fair. Until the 20th century, his work was mainly known from the edited highlights from his letters published by the Royal Society. Moreover, when Edmund Halley controlled the Philosophical Transactions, Van Leeuwenhoek's work was not included, and he published his work in Dutch books. In 1934, a Dutch Committee began publishing the Collected Letters in English and modern Dutch.

Objectives: Experiments with insect corneas, as described in the Collected Letters, were repeated and then discovered to have not been published by the Royal Society, although submitted. This poster presents results obtained with bee and dragon-fly eyes, using Van Leeuwenhoek's methods.

Methods: Eyes from dead bees and dragon-flies were dissected and cleaned. The corneas were mounted on coverslips and photographed through facsimiles of Van Leeuwnhoek microscopes with natural, LED and candle-light.

Results: Results similar to Van Leeuwenhoek's were obtained. As with previous experiments, small points were not described, but could be worked out. The Royal Society sometimes asked how something was done, and occasionally he preferred not to say, but he usually explained. Many of the letters published (and edited) by the Royal Society do not include full descriptions of methods, the authors apparently being more enthusiastic about their results. Of course, some of his apparent reticence might have been fear of plagarism after Hartsoeker claimed the discovery of sperm, having seen them at Van Leeuwenhoek's house

PT142 Importance of measurement uncertainty in the determination pH value in culture media

Anita Rakic[1]

[1]*Public Health Institution of Split-Dalmatian County, Split, Croatia*

Background: Testing laboratories determine control procedures of culture media prepared from dehydrated base as well as the ways to control technological processes of preparation according to EN ISO 11133:2014 and EN 12322:2008. An accreditation of a laboratory conducting certain tests contributes to improving the quality of each laboratory. Therefore, when we say that we did an analysis in an accredited laboratory, we can have confidence in the quality of the lab's work because the work of that laboratory is carried out in accordance with certain quality norms.

Objectives: The aim of the study was measure the pH using a pH meter according to ISO 7218 and ISO 8199 in the preparation of culture media the Tryptic Soy Agar (TSA).

Methods: The pH value is determined using direct method with pH-meter, 827 pH lab. The pH standard quantification is carried out by use of calibration curve and results are expressed in pH units. The validation of the method is executed over 10 days, with three 2-level neasurements per day. For the purpose of assessing uncertainty in measurement and calculation of the combined and extended uncertainty the data obtained from the method validation (reproducibility, accuracy), calibration curve, of preparation culture media TSA from dehydrated commercial formulations.

Results: The comparison of relative standard uncertainty makes it evident that the most significant input to measurement uncertainty derives from the calibration curve and long-term reproducibility. All these are mechanisms that have an impact on the improvement and quality assurance of work in microbiological laboratories.

PT143 Abdominal Organ Transport Fluid Testing

Mario Aramouni[1], Ijeoma Nwafor[1], Ruhi Siddiqui[1]

[1]*Public Health England, United Kingdom*

Background: Infectious complications are amongst the major causes of morbidity and mortality in patients undergoing solid organ transplantation. Contamination of the graft with subsequent recovery of micro-organisms from the organ transport fluid (TF) may represent a source of early post-surgical infections in transplant recipients. The TF used to preserve the graft offers favorable conditions in which microorganisms can survive. The true clinical impact of microbiological contamination during abdominal organ retrieval remains unresolved, with isolation rates varying widely (very low to up to 40%).

Objectives: To develop an agreed methodology for culture of abdominal organ TF, including the reporting of results, so as to contribute to improved and safer practices in abdominal organ transplantation. To improve consistency and harmonize practices across the UK.

Methods: The UK SMI B62 standard was developed and reviewed through a wide consultation process with users and other stakeholders. The process follows the AGREE tool and the resulting document reflects best evidence based practice. Where evidence is lacking, the document includes recommendations based on the established working group consensus decisions. The process used is accredited by The National Institute for Health and Care Excellence (NICE) and certified to ISO 9001.

Results: The laboratory best minimum practice for microbiological investigation of abdominal organ transport fluid was developed based on currently available microbiological tools, observing the requirements of the organ donation and transplantation pathway in the UK. It offers guidance on reporting and notification of results to NHS Blood and Transplant Organ Donation and Transplantation, as per national requirements.

PT145 Microbial Pursuit: an interactive and collaborative game-based method to learn microbiology

Isabel Murillo[1]

[1]*University of Bristol, Cellular and Molecular Medicine, Bristol, United Kingdom*

Background: Playing games is an invaluable and widely-used educational tool in both primary and secondary schools. Sadly, it seems to be largely forgotten in Higher Education.

This pedagogical project involves the creation and playing of a board game called Microbial Pursuit. It resembles Trivial Pursuit with questions on Microbiology as a method of unit revision. The "hardware" (board, dice, etc) were provided by the teacher and the questions and answers were made by the students.

Objectives: It is therefore in both the making and in the playing of the game that learning takes place. From the outset students were aware they were creating a competitive game to play with and against their colleagues. Both the competitive and collaborative elements to the activity helped students engage with it. This project proposes the perfect game to convert tutorials into pedagogical and enjoyable sessions.

Methods: Students worked in groups of 6 over several sessions. Each group was asked to write a number of questions on their specific Microbiology topic. Students used class handouts, laboratory practical material and Google as a source of information. Students created different types of questions and where possible questions were similar in style to those the students encounter in their examinations.

Results: To evaluate the effectiveness of the game the same questionnaire were filled in before and after the game. The oral and written feedback received from students has been positive and very encouraging. Microbial Pursuit has proved to be an enjoyable and successful a method of unit revision.

PT146 Do-It-Yourself-Biology - Genetic Engineering for Everyone?

Birgit Schoenig[1], Anke Stein[1]

[1]*Federal Office of Consumer Protection and Food Safety, Genetic Engineering, Berlin, Germany*

Background: In recent years, public interest in molecular biology and microbiology has increased. A growing part of the public is interested in creating new microorganisms with novel features and in participating in scientific progress. Experimental kits covering this need for (the so called) Do-it-Yourself (DIY) Biology are provided by commercial vendors via the internet.

However, European regulations on the use of genetically modified microorganisms (GMMs) and on infection prevention restrict the free use of GMMs and potentially human-pathogenic microorganisms and set regulatory and organisational limits, which need to be respected. Thus, members of the public need to be aware of these restrictions in order not to carry out experiments which might endanger their health and in order not to violate European or national law.

Results: The poster presents recent developments in the DIY biology movement, summarizes the regulatory background of DIY biology, discusses efforts to prevent the importation of DIY biology kits with pathogenic and/or genetically modified microorganisms and offers ways ahead, how members of the public interested in DIY biology can safely and legally carry out these experiments.

PT147 The role of adherent and invasive e. coli in pediatric crohn's disease, cause or effect

Nojoud Faqerah[1]

[1]*United Kingdom, Bacteriology, glasgow, United Kingdom*

Background: Crohn's disease (CD) is a form of inflammatory bowel disease and is likely due to an inappropriate immune response against enteric microbes in genetically susceptible individuals. Adherent-invasive *E. coli* (AIEC) and their mechanisms in adhesion to and invasion of intestinal epithelial cells and the ability to survive autophagy within macrophages support their implication in the pathogenesis of CD.

Objectives: Studying the role of Adherent-invasive *E. coli* (AIEC) strains in initiation of paediatric Crohn's disease (CD), and the impact of Exclusive enteral nutrition (EEN) and diet on AIEC behaviour in active paediatric CD and in healthy people.

Methods: *E. Coli* strains were isolated from culturing stool samples of CD patients before and after EEN treatment to study the phenotype and genotype changes in *E. coli* population. This is achieved by using Biolog phenotype microarrays, colicin sensitivity spot tests for characterization of *E. coli* strains, and metagenomic analysis for genome study. 35 samples were investigated (CD, n=25; Non-IBD, n=5; Healthy, n=5) by using DNA sequencing on Illumina sequencer.

Results: From the preliminary results, there were changes in three out of five Crohn's disease patients in bacterial species according to 16s rRNA sequencing, and changes in *E.coli* strains as stated in colicin sensitivity results before, during and at the end of EEN. The results of metagenomic data are under processing. Studying a large number of patients by analysing the metagenomic data will provide a comprehensive understanding in changes of *E. coli* population in CD during relapse and remission.

PT148 Gut microbiota ecology during Campylobacter infection in chickens: a description of community changes via metataxonomic characterization.

Ilaria Patuzzi[1;2], Eleonora Mastrorilli[1], Veronica Cibin[3], Alessia Tiengo[3], Sara Petrin[1], ALESSANDRA LONGO[1], Salvatore Catania[4], Federica Gobbo[4], Antonia Ricci[5], Carmen Losasso[1]

[1]*Istituto Zooprofilattico Sperimentale delle Venezie, Microbial Ecology Unit, Legnaro, Italy*
[2]*University of Padova, Department of Information Engineering, Padova,*
[3]*Istituto Zooprofilattico Sperimentale delle Venezie, National reference centre for salmonellosis, Legnaro, Italy*
[4]*Istituto Zooprofilattico Sperimentale delle Venezie, Avian Medicine Laboratory, Legnaro, Italy*
[5]*Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Italy*

Background: Campylobacter is the most important foodborne zoonotic agent in EU with broiler being the principal reservoir for human infection. Control measures to prevent *Campylobacter* colonization at farm level have not been effective so far. Thus, deeper knowledge on *Campylobacter* ecological interaction within the gut is needed.

Objectives: Gut microbiota composition between infected and non-infected broilers was compared to investigate dynamics possibly favouring *Campylobacter* colonization of the gut.

Methods: Four broiler farms, half of them being positive for *Campylobacter*, were enrolled. Caecal samples were collected from each farm at different time points (7th, 14th, 18th, 21th and 28th day). V3-V4 16S rRNA gene regions were amplified and sequenced using HiSeq2500 Illumina platform. OTU table was investigated to describe community diversity in terms of alpha and beta indices. Additionally, a differential abundance analysis was performed to check for differences in temporal evolution of the microbial community between negative and positive farms and among the positive farms. Finally, population dynamics was investigated through the reconstruction of inter-genera interaction networks.

Results: Alpha diversity was significantly different between negative and positive samples. Moreover, beta diversity analysis showed that positive farms exhibited higher similarity already in the pre-infection phase and lower similarity with the negative farms. This could suggest that a favourable condition to *Campylobacter* was already in place before infection arose. *Lactobacillus*, *Anaerotruncus* and *Clostridium* were found to inhibit *Campylobacter* gut colonization; *Faecalibacterium*, *Bifidobacterium* and *Ruminococcus* to enhance it. Microbial interaction networks could be used to delineate new strategies against *Campylobacter* infections in poultry.

PT149 The role of the gut microbiota in Clostridium difficile infection.

Amy Irvine[1], Andrew Free[2], Olga Moncayo[3], Luke Tysall[3]

[1]University of Edinburgh, Usher Institute of Population Health Sciences and Informatics, Edinburgh, United Kingdom
[2]University of Edinburgh, Quantitative Biology, Biochemistry & Biotechnology, Edinburgh, United Kingdom
[3]NHS Lothian, Edinburgh, United Kingdom

Background: *Clostridium difficile* infection (CDI) is a leading cause of healthcare associated diarrhoea with considerable morbidity, mortality and economic burden. The gut microbiota is influenced by factors such as geography, diet, medication and age and plays a protective role against CDI via colonisation resistance which can be weakened or lost with antibiotic use. As the gut microbiota plays such an important role in protection from CDI it may be possible to exploit these changes and use them for diagnostic or predictive purposes. With recent denoising techniques, it is now possible to study exact sequence variants (ESVs), sequences clustered at 100% similarity which have the potential to uncover more accurate and reliable biomarkers compared with operational taxonomic units (OTUs).

Objectives: This study aims to discover if there are any links between clinical and lifestyle factors and, disease severity/outcome using gut microbiota composition as a biomarker while comparing the use of OTUs and ESVs. The predicitve ability of identified biomarkers will then be assessed.

Methods: Faecal samples were collected from toxin-positive and equivocal CDI patients. DNA was extracted and the V4 region of the 16S rRNA gene amplified using the 515F and 806R primers, and sequenced via Illumina MiSeq. The sequencing data were analysed with both the QIIME1 and QIIME2 pipelines and clustered to 97% OTUs and ESVs respectively.

Results: The results show high inter-individual variability across the study; based on this we will discuss the feasibility of using gut microbiota biomarkers as indicators of disease severity and patient outcome prediction.

PT150 Microbiota of the gut-lymph node axis: Depletion of mucosa-associated segmented filamentous bacteria and enrichment of Methanobrevibacter by Colistinsulfate and Linco-Spectin in pigs

Benjamin Zwirzitz[1;2], Beate Pinior[3], Barbara Metzler-Zebeli[4], Monika Handler[1], Kristina Gense[1], Christian Knecht[5], Andrea Ladinig[5], Monika Dzieciol[1], Stefanie U. Wetzels[1;2], Martin Wagner[1], Stephan Schmitz-Esser[6], Evelyne Mann[1]

[1]*Institute of Milk Hygiene, Milk Technology and Food Science, Department for Farm Animal and Public Health in Veterinary Medicine, Vienna, Austria*
[2]*FFoQSI GmbH, Austria*
[3]*Institute of Veterinary Public Health, Department for Farm Animal and Public Health in Veterinary Medicine, Vienna, Austria*
[4]*Institute of Animal Nutrition and Functional Plant Compounds, Department for Farm Animal and Public Health in Veterinary Medicine, Vienna, Austria*
[5]*University Clinic for Swine, Vienna, Austria*
[6]*, Department of Animal Sciene, Ames, United States*

Background: In-feed antibiotics alter the gut microbiome and consecutively might also affect translocation processes of microorganisms to lymphatic tissues. As a result, intestinal microbiota and the host immune system could fall into an imbalanced state, making the host susceptible to recurrent infections and dysbiosis.

Objectives: By providing the first comparative study of microbial communities in pig feces, ileum, and ileocecal lymph nodes under the influence of in-feed antibiotics, we aim to better understand the variant effects of antibiotics on the microbiome in these specific tissues, which is of vital importance for animal production and health.

Methods: We used 16S rRNA gene high-throughput sequencing to characterize the microbial communities of the analyzed tissues in a 3-week feeding trial. Furthermore, we also investigated the microbiome of ileocecal lymph nodes by cultivation, generating 95 isolates, and by sequencing the metatranscriptome of a single lymph node sample.

Results: The *Proteobacteria*-dominated lymph node microbiome represented a sub-fraction of the gut microbiome with a significant lower diversity compared with ileum and feces. In each analyzed tissue, we identified phylotypes susceptible to antibiotic treatment that hold profound impacts on the host physiological and immunological state, with the lymph node microbiome being affected by antibiotics to a lesser extent compared with feces and ileum. RNA-sequencing of a lymph node unveiled expressed transcripts for bacterial metabolic core processes like amino acid and carbohydrate metabolism, therefore proving the metabolic activity of bacteria in lymph nodes. Our results indicate that pathogenic bacteria could escape antibiotic treatment, after translocation to lymph nodes.

PT151 Lifestyle Influences the Structure and Predicted Function of the Gut Microbiota in Nomadic Fulani

Ayorinde Afolayan[1], Funmilola Ayeni[2], Christoph Hoegenauer[3]

[1]Global Health Research Unit, Department of Pharmaceutical Microbiology, Faculty of Pharmacy, University of Ibadan, Ibadan, Nigeria
[2], Department of Pharmaceutical Microbiology, Faculty of Pharmacy, University of Ibadan, Ibadan, Nigeria
[3]Division of Gastroenterology and Hepatology, Department of Internal Medicine, Medical University of Graz, Graz, Austria

Background: The human gut microbiota plays a major role in the normal functioning of the human body. Lifestyle affects the gut microbiota. However, its contribution on the gut microbiota of vulnerable populations in Nigeria is relatively unknown.

Objectives: This study focused on the effect of nomadic lifestyle of the Fulanis on their gut microbiota.

Methods: Human faecal samples were obtained from nomadic Fulanis (Pabaman-shanu village, Plateau State; n = 28) and non-Fulanis (Lamingo, Jos, n = 22). The DNA of all samples were extracted, followed by PCR amplification of the V4 region of 16S rRNA gene, library preparation, and sequencing with Illumina MiSeq. From the generated raw sequence reads, the gut microbiota of Fulanis and non-Fulanis were compared for diversity by QIIME, functional profile by PICRUSt, unique structural and predicted functional biomarkers by LEfSe, and relative abundance of predicted pathogens by BugBase.

Results: The Fulanis had lower alpha diversity. Differential phyla include Bacteroidetes (Prevotella) in the Fulanis, and Firmicutes (Ruminococcus) in the non-Fulanis. Lipopolysaccharide biosynthesis, Vibrio cholerae pathogenic cycle, and Glutathione metabolism were enriched in the Fulanis, while methane metabolism and fructose and mannose metabolism were overrepresented in the non-Fulanis, suggesting the impact of dietary and hygiene habits on the gut microbiota. A higher abundance of potential gut pathogens was observed in the Fulani. The reduced gut microbiota alpha diversity is a reflection of the Fulani's lifestyle, which increases vulnerability to diseases. Differential taxa and metabolic pathways could serve as potential markers for health risk of similar study groups.

PT152 Assessing biogas.producing microbial communities with MinION-based sequencing

Adriel Latorre-Pérez[1], Christian Abendroth[2;3], Justus Hardegen[2], Patrice Ramm[4;5], Olaf Luschnig[6], Claudia Simeonov[2], Javier Pascual[1], Cristina Vilanova[1], Manuel Porcar[1;7]

[1]Darwin Bioprospecting Excellence S.L., Paterna, Spain
[2]Robert Boyle Institut e.V., Jena, Germany
[3]Dresden University of Technology, Dresden, Germany
[4]Leibniz Institute of Agricultural Engineering and Bio-economy e.V. (ATB), Potsdam, Germany
[5]Institute of Agricultural and Urban Ecological Projects affiliated to Berlin Humboldt University (IASP), Germany
[6]Bio-H2 Umwelt GmbH, Jena, Germany
[7]I2SysBio (Institute for Integrative Systems Biology), Paterna, Spain

Background: In the last years, metagenomic techniques have led to a better understanding of the microbial communties involved in biogas production. However, there is a lack of information about how different factors (substrate election, co-digestions, etc) could affect the biogas microbiome. Third generation sequencing platforms, such as Oxford Nanopore Technologies, are a promising tool for the monitorisation of these communities due to their low cost, portability, and real-time generation of the sequencing data.

Objectives: This work aimed at testing new approaches for the analys of methanogenic communities in biogas digesters, as well as correlating different reactor configurations with population shifts.

Methods: The 16S rRNA gene was full-length amplified and sequenced with a MinION portable sequencer. Data were then analysed with custom bioinformatic and biostatistical tools.

Results: MinION sequencing proved to be a suitable tool for studying the microbiomes associated to biogas production. Our data unveiled the influence of particular operational conditions on the methanogenic communities, and allowed us to propose novel strategies to improve biogas yield and quality. This work paves the way towards a real-time monitorisation of process performance on the basis of the underlying microbial communities.

PT153 Screening of a panel of dominant gut anaerobes for putative health impacts

Elena Conti[1], Sylvia Duncan[1], Alan Walker[1]

[1]*The Rowett Institute, Aberdeen, United Kingdom*

Background: The human gut microbiota exerts important roles in human health. One of the main drivers of its composition and activity is host diet. A high-fibre diet may be protective against colorectal cancer (CRC), by promoting the bacterial production of beneficial compounds. Conversely, a high-fat diet may increase secondary bile acids formation by gut commensals, promoting CRC risk. Although some of the links between diet, the gut microbiota and health are known, little is understood about the potential health impacts of most individual gut bacteria species.

Objectives: To assess the potential health impacts of a representative panel of thirty-three diet-responsive human gut anaerobes, in particular traits such as antibiotic resistance, virulence, bile salt conversion and potential production of novel antimicrobials.

Methods: The thirty-three gut anaerobe genomes were mined for presence of genes encoding putative antibiotic resistance, virulence factors, bile salt hydrolases, and secondary metabolite operons. *In silico* data were compared/verified with *in vitro* analyses including antibiotic resistance tests, antimicrobial activity against model gut pathogens using overlay assays, and bile salt tolerance testing.

Results: The panel of gut anaerobes tested did not show wide-scale antibiotic resistance and virulence traits. Certain gut anaerobes possessed the ability to inhibit the growth of gut pathogens including *Clostridium difficile*, and may have the genetic repertoire to form novel antimicrobial compounds. Many potentially beneficial gut anaerobes were less tolerant to bile than opportunistic pathogens from the *Enterobacteriaceae* family. Cumulatively, these results enhance our knowledge about the potential health impacts of dominant and diet-responsive human gut anaerobes.

PT154 The nose-brain axis - The correspondence of the nasal microbiome and olfactory function

Christina Kumpitsch[1], Kaisa Koskinen[1;2], Florian Fischmeister[3], Christine Moissl-Eichinger[1;2], Veronika Schöpf[3]

[1]Medical University Graz, Department of Internal Medicine, Graz, Austria
[2]BioTechMed Graz, Graz, Austria
[3]University of Graz, Institute of Psychology, Graz, Austria

Background: The loss of smell is an incisive event, caused by mechanical impact, infection, or disease, or occurs subtly during ageing. It affects psychological, social and behavioral performance and impacts the quality of life tremendously. The loss of olfaction goes along with a reorganization of the functional network structure of the human brain. The ability to smell is mediated by olfactory neurons in the ceiling of the nose, the olfactory mucosa, which is inhabited by numerous microorganisms. In general, the human body is associated with trillions of microorganisms (the microbiome) which are intertwined with human health, disease and maybe even behavior. The microbiome communicates with the human body cells and affects functions of the brain and other tissues.

Objectives: We aim to decipher the role of the nasal microbiome in olfactory function, dysfunction and regain.

Methods: Neuroimaging biomarkers and olfactory function are being correlated with microbiome measures such as diversity, abundance and functional information (metatranscriptomics). Furthermore, scanning electron microscopy and metabolomics will be used to further investigate the interaction of the nasal microbiome with olfactory performance.

Results: Our completed pilot study showed that the microbial community composition in the olfactory mucosa mirrors the capability to smell. Volunteers with impaired smelling capacity showed an increase of specific (anaerobic) microbial groups, such as butyrate producers. Understanding the microbial community in the olfactory mucosa will increase therapeutic opportunities, and possibly allow monitoring and predicting smell therapy success in future.

PT155 Signature taxa indicate the storability of sugar beets before harvest

Peter Kusstatscher[1;2], Tomislav Cernava[2;3], Karsten Harms[4], Johann Maier[4], Herbert Eigner[5], Gabriele Berg[2], Christin Zachow[1]

[1]*ACIB GmbH, Graz, Austria*
[2]*Graz University of Technology, Institute of Environmental Biotechnology, Graz, Austria*
[3]*Roombiotic GmbH, Graz, Austria*
[4]*Südzucker AG, Mannheim, Germany*
[5]*Agrana Research & Innovation Center, Tulln, Austria*

Background: Sugar beets are the main source of sugar in temperate regions around the globe. Due to extended campaign durations, sugar beets are stored for an average of 45- 60 days after harvest. Microbial degradation during this time decreases the sugar content substantially.

Objectives: To identify disease induced microbiome changes in the field and during storage signatures in the microbiome of beets were studied.

Methods: Using a barcoded amplicon sequencing approach the bacterial and fungal microbiome of healthy sugar beets in the field as well as in storage was accessed and compared to the microbiome of decaying sugar beets. Cultivation-dependent methods were applied to complement the picture.

Results: Highly significant changes in the beet microbiome were observed to be dependent on the health status. Fungal taxa such as *Plectosphaerella* and *Vishniacozyma* dominating the microbiome of healthy beets were replaced by *Candida*, *Fusarium* and *Penicillium* in decaying beets. Furthermore, an increased fraction of Gram-positive *Lactobacillales* was observed in decaying sugar beets. The taxonomic changes in the fungal microbiome were accompanied by changes in trophic specialization e.g. increased saprotrophic fraction. Using the generated knowledge new advanced biocontrol strategies can be developed. By maintaining the health-related microbiome an increased storability of sugar beets can be assured.

PT156 Impact of the "first" domain of life - improved protocols for detection and quantification of archaeal signatures from the human microbiome

Marcus Blohs[1], Pausan Manuela [1], Koskinen Kaisa[1], Mahnert Alexander[1], Christoph Hoegenauer[2], Schmitz-Streit Ruth[3], Christine Moissl-Eichinger[1]

[1]*Medical University Graz, Internal Medicine, Graz, Austria*
[2]*Medical University Graz, Division of Gastroenterology and Hepatology, Graz, Austria*
[3]*Institut Für Allgemeine Mikrobiologie, Kiel, Germany*

Background: Humans are host to a high variety of microorganisms, including archaea, bacteria, eukaryotes, and viruses. During the last decades it became apparent that the interplay between host and its microbiome is crucial and influences host health and well-being. While the role of bacteria is extensively studied to date, we know rather little about diversity and function of the other domains. This includes also the “first” domain of life (if arranged alphabetically), namely the archaea. Even though Archaea are regarded as keystone species in the gastrointestinal tract with unique metabolic capacities (e.g. methane production), they remain understudied in microbiome projects. This is due to methodological pitfalls, leading to an underestimation of archaeal presence, abundance and importance.

Objectives: We aim to develop and improve protocols that will help to reveal the important role of Archaea in the microbiome of the human body in context of their clinical relevance.

Methods: Here, we propose improved, archaea-specific primers for 16S rRNA gene detection and quantification, as well as new approaches for isolation and cultivation of methanogenic archaea from the gut.

Results: By improved primer combinations we were able to confirm the presence of Archeaea on all conceivable human body sites, accounting for up to 50% of the microbial diversity in the nose and appendix. We also established a pipeline for the isolation and cultivation of methanogenic archaea from human stool samples, with the intention to analyse genomic and metabolomic traits of archaea that are puatatively involved in microbial dysbiosis.

PT157 Glycan-specific natural antibodies repertoire in aGalT-KO mice: a function of gut microbiota diversity?

Sara Olivera-Ardid[1], Daniel Bello-Gil[2], Christophe Audebert[3], Nicolai Bovin[4], Nailya Khasbiullina[4], Rafael Mañez[5]

[1]*IDIBELL, Hospitalet de Llobregat, Spain*
[2]*RemAb Therapeutics, Hospitalet de Llobregat, Spain*
[3]*Institut Pasteur de Lille*
[4]*Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow,*
[5]*El Hospital Universitario de Bellvitge, Hospitalet de Llobregat, Spain*

Background: Gut bacteria are known to have a significant role in regulating the innate and adaptive immune homeostasis. Alterations of this microbial composition have been associated with several diseases, including autoimmune and inflammatory conditions. Despite this, it is not clear whether commensal gut microbiota can modulate the systemic immunity, and how circulating elements of the host immune system could regulate microbiome.

Objectives: We aimed to study and set relations among the microbiota diversity and repertoire of circulating natural anti-carbohydrate antibodies in αGalT-KO mice during the first 7 months of animal life.

Methods: The diversity and abundance of specific taxons were performed by metagenetic high-throughput sequencing. The repertoire of natural antibodies was obtained by printed glycan-array technology and associated with the microbial diversity by metagenome-wide association studies.

Results: The orders *Clostridiales, Bacteriodales, Lactobacillales* and *Deferribacterales* seem to be associated with the development of the repertoire of natural anti-glycan antibodies in GalT-KO mice. The main changes in microbiota diversity were related to important changes in levels and repertoire of natural anti-glycan antibodies. Additionally, significant positive and negative associations between the trend followed by specific anti-glycan antibodies and gut microbiota were found. Regarding individual differences, the gut microbiota and as a consequence, the repertoire of natural anti-glycan antibodies was not identical among the examined animals. We also found redundancy in different taxa associated with the development of specific anti-glycan antibodies. Differences in microbiota composition did not, therefore, necessarily resulted in alterations to the overall functional output of the gut microbiome of GalT-KO mice.

PT158 ndm1 gene detection among the carbapenem resistant escherichia coli and klebsiella pneumoniae isolated from a children's hospital in kathmandu

Binod G C[1], nim raj sapkota[1], binod rayamajhee[2], Santosh Khanal[1]

[1]*National College, Department of Microbiology, Kathmandu, Nepal*
[2]*Kathmandu Research Institute for Biological Sciences, Lalitpur, Nepal*

Background: Infections with carbapenem resistant Enterobacteriaceae (CRE) are increasingly reported from healthcare facilities in Nepal and around the world. *bla*$_{\text{NDM-1}}$ along with several other genetic elements with multiple resistant genes are harbored and transmitted between Gram negative bacteria.

Objectives: To detect New Delhi metallo-β- lactamase 1 (*bla*$_{NDM-1}$) gene among carbapenemase producing *E. coli* and *K. pneumoniae*

Methods: The organisms were isolated, identified and antimicrobial susceptibility test was done following CLSI Guidelines 2014. Modified Hodge Test was performed to detect carbapenemase production and NDM-1 producers were confirmed by genetic analysis.

Results: During 12 months period of this study from November 2017 to November 2018, total of 1705 samples were processed. Urine, blood, swab, pus and CSF samples were collected from the patients visiting Children's Hospital in Kathmandu. Carbapenem i.e. imipenem (84.2 %) and meropenem (80.3 %) were found to be most effective antibiotics against the isolates. 48.1 % of the isolates were found to be MDR. From 121 *E. coli* and 45 *K. pneumoniae* isolated, 45 *E. coli* and 24 *K. pneumoniae* were screened as possible carbapenemase producers in this study. Nine isolates of *E.coli* and 5 isolates of *K. pneumoniae* were confirmed as carbapenemase producers by Modified Hodge Test. Furthermore, PCR was carried out among the MHT positive organisms for the detection of *bla*$_{\text{NDM-1}}$ gene. Genetic analysis of MHT positive isolates showed 4 out of 9 *E.coli*, 3 out of 5 *K. pneumoniae* to be NDM-1 producers.

PT159 Effect of plant development stage on rhizosphere communities of Groundnut (Arachis hypogaea)

Ankit Hinsu[1], Prakash Koringa[2], Ashvin Dumadiya[1], Rameshkumar Kothari[1]

[1]*Saurashtra University, Department of Biosciences, Rajkot, India*
[2]*Anand Agricultural University, Department of Animal Biotechnology, Anand, India*

Background: Rhizosphere, a zone nearby the roots, is the hotspot for plant-microbe interaction. Rhizospheres microbes are an essential component of plant development and plant health. Both biotic and abiotic factors regulate the rhizosphere microbes. Hence, it is essential to understand the factors that control microbial presence and functions.

Objectives: To evaluate the soil microbial diversity during different phases of plant development.

Methods: Soil samples were collected from bulk and rhizosphere of groundnut at an approx interval of 15 days including samples during pre-sowing and post-harvest. DNA was extracted from soil using QIAGEN PowerSoil DNA kit. V3-V4 region of 16s was amplified using 341F and 785R primers and sequenced on Illumina MiSeq using 250bpx2 chemistry. Data was quality-filtered using Prinseq-lite and paired-end reads were merged using Pandaseq. Further analysis was carried out using QIIME 1.9.1 by closed-reference OTU picking at 97% identity.

Results: Approximately 4.5 million good quality-reads were analysed in QIIME resulting into 1,751 OTUs with minimum abundance 0.005%. Higher diversity was observed in bulk soil compared to rhizosphere soil as evident by alpha-diversity indices like Shannon index, simpson index and PD whole tree. PCA analysis revealed difference in composition among bulk soil and rhizosphere soil; while pre-sowing and post-harvest samples were similar to bulk soil. Clear demarcation was observed first rhizosphere sample (germination phase), last rhizosphere sample (matured plant) and other rhizosphere samples. *Pseudomonas*, *Bacillus*, *Kaistobacter* and around 80 other genera were observed to significantly vary (ANOVA FDR $p<0.05$) among different time-point sample of Rhizosphere.

PT160 Next-Generation Sequencing reveals the relationship between the gut microbiota and the food substrate in the polyphagous fruit fly Bactrocera tryoni

Rajib Majumder[1;2], Brodie Sutcliffe[1], Phil W. Taylor[1], Toni Chapman[1;2]

[1]*Macquarie University, North Ryde, Australia*
[2]*Elizabeth Macarthur Agricultural Institute, Menangle, Australia*

Background: The gut microbiome is essential to the insect, a necessary source of various nutritional benefits to the host. While recent studies have shown that diet is a crucial modulator of the gut microbiota composition, very little is known about the dynamics underpinning microbial acquisition from natural food sources

Objectives: We aimed to answer two questions: first, how does gut microbial composition vary across Queensland fruit-fly, *Bactrocera tryoni* larvae and its various host fruit source? Second, how does the fruit source affect the bacterial community in the gut of wild larvae?

Methods: We were compared the microbiome of the wild larvae from the polyphagous fruit-fly pest *B. tryoni* across six different food substrates (i.e., sapodilla, hog plum, pomegranate, green apple, and quince) from the north- to south-east Australia. After sterilization, DNA extraction and PCR of the larvae and fruits samples, gut bacterial analysis was performed based on V1-V3 region of the 16s r RNA gene using Next-Generation Sequencing (NGS) on the Illumina MiSeq platform

Results: Our study is suggesting that the gut bacterial community structure of *B. tryoni* larvae is related less to the host fruits (diet) microbiome but more to the bacterial vertical transfer from the mother and the larval ecology. To our knowledge, this study is the first NGS study to analyze both the microbiome in the gut of the wild larvae and its host fruits. Finally, this study is adding to the growing knowledge in the area of the microbial ecology of the fruit-fly

PT161 Comprehensive analysis of the spectacle bacteriota using 16S rRNA gene sequencing

Birgit Fritz[1], Severin Weis[1], Focke Ziemssen[2], Siegfried Wahl[3], Markus Egert[1]

[1]*Institute of Precision Medicine, Furtwangen University, Villingen-Schwenningen, Germany*
[2]*Centre for Ophtalmology, Eberhard-Karls-University, Tuebingen, Germany*
[3]*ZEISS Vision Science Lab, Institute for Ophthalmic Research, Eberhard-Karls-University Tuebingen, Germany*

Background: Although widespread in population, little is known about the microbial contamination of spectacles and their hygienic relevance. Aerobic cultivation analyses from our group [1] revealed that spectacles are significantly contaminated with bacteria, in particular with staphylococci. However, aerotolerant anaerobes, slow growing and yet-uncultivated bacteria were probably discriminated with this approach.

Objectives: To obtain a more comprehensive picture of the community profile, we performed a cultivation independent analysis of the bacteriota on worn spectacles using massive 16S rRNA gene sequencing.

Methods: 35 spectacles from university staff and students were swab-sampled at three sites (nosepads, glasses, earclips). We sequenced the V3 – V4 region of the 16S rRNA gene on an Illumina MiSeq platform using an optimized library preparation protocol for samples with low DNA content.

Results: After rarefication, 22 phyla and 663 genera of bacteria were detected. *Actinobacteria* (42%), *Firmicutes* (39%) and *Proteobacteria* (14%) were the dominant phyla. At genus level, only a few taxa dominated the bacteriota: *Staphylococcus* (28%), *Propionibacterium* (21%), *Corynebacterium* (11%), *Lawsonella* and *Streptococcus* (4%, each). Statistical analyses revealed a significantly higher bacterial diversity on the glasses compared to nosepads and earclips.
Our study represents the first comprehensive insight into the cultivation independent composition of the bacteriota of worn spectacles. Dominated by aerobic and anaerobic bacteria of mostly human skin and epithelia origin and clearly including potentially pathogenic ones, spectacles may play a role as fomites, especially in clinical environments.

[1] Fritz B et al. (2018). PLoS ONE 13 (11), e0207238. DOI: 10.1371/journal.pone.0207238.

PT162 Lung microbiome dynamics during pneumonia and antibiotic therapy

Ana Elena Pérez Cobas[1;2], Christophe Ginevra[3;4], Christophe Rusniok[1;2], Sophie Jarraud[3;4], Carmen Buchrieser[1;2]

[1]*Institut Pasteur, Paris, France*
[2]*CNRS UMR 3525, Paris, France*
[3]*Centre National de Référence des Légionelles, Bron, France*
[4]*Centre International de Recherche en Infectiologie, Lyon, France*

Background: Despite the explosion of microbiome analyses, very little is known about the pulmonary microbiome, especially regarding infectious diseases, although for example pneumonia represents a major public health problem with a high rate of morbidity and mortality worldwide. Analyses of other habitats revealed that microorganisms participate in the immune system equilibrium and in protecting against pathogens. However, the role of the lung microbiome during infection is poorly understood.

Objectives: We aimed to characterize the evolution of the lung microbiome and mycobiome during infection and antibiotic therapy. We used the infection by the bacterium Legionella pneumophila as a model to study the lung microbiome during pneumonia.

Methods: We collected broncheoalveolar lavages from infected individuals during hospitalisation for a longitudinal study. The bacterial and fungal diversity of the samples were characterized by high-throughput Illumina sequencing of the 16S rRNA gene and the ITS region. A comparison of the lung microbiome composition during pneumonia to the one of healthy individuals1 was performed to identify microorganisms associated with dysbiosis or those that might be protective.

Results: The bacterial lung microbiome during infection and antibiotic treatment represents a disturbed state with a low diversity, high abundance of the pathogen, prevalence of opportunistic microorganisms and poor representation of commensal bacteria. However, the mycobiome remains more stable compared to the microbiome but possible cooperation within and between both communities were identified. These interactions may be important for the restoration of the microbiome and the clinical evolution of the disease.1- Segal et al., (2016) Nat Microbiol. 1:16031.

PT163 Effects of probiotic supplementation on gut microbiota and treatment of mild alcoholic liver disease

Byung-Yong Kim[1], Min-Jung Kwak[1], Seonbin Choi[1], Kyoung-Hee Cho[1], Jongsik Chun[1,2], KI TAE SUK[3]

[1]*ChunLab, Inc., Microbiome Research Centre, Korea, Rep. of South*
[2]*Seoul National University, School of Biological Sciences, Seoul, Korea, Rep. of South*
[3]*Hallym university, College of medicine, Chuncheon, Korea, Rep. of South*

Background: Alcohol drinking cause abnormal overgrowth of harmful bacteria within bowel and disruption in the gut barrier. Consequently, it may promote the translocation of lipopolysaccharide (LPS) in the intestine. Probiotics has been reported to restore gut microbiome by reducing gut-derived microbial LPS in patients with alcoholic liver disease (ALD)

Objectives: To elucidate the relationship between alcohol drinking and changes of gut microbiota, and its involvement in the pathogenesis of alcohol liver disease, we performed microbiome analysis in mild ALD patients with probiotic supplementation. Also we tested gut microbiota modulation could be a target for the treatment of ALD in this study.

Methods: 101 patients with ALD (probiotics: 53 and placebo: 48) and normal patients were prospectively randomized to receive the 7 days of *Lactobacillus rhamnosus* and *Lactobacillus acidophilus* at 120mg/day or placebo. Liver function and LPS level were evaluated by enzyme-linked immunosorbent assay (ELISA). The relative abundance of gut bacterial changes of ALD patients were determined with NGS sequencing of 16S rRNA genes, and compared between before and after probiotics treatment for 7 days.

Results: Seven-days of probiotics supplementation were associated with restoration of gut microbiota, suggesting that probiotics might be effective in the treatment of alcoholic hepatitis by gut-liver axis.

PT164 Systemic pro- and anti-inflammation were related to dysbiosis in gut microbiota after Mycobacterium Tuberculosis infection in HIV-uninfected humans

Shiang-Fen Huang[1;2], Ying-Ying Yang[1;2], Wei-Juin Su[1], Chang-Phone Fung[1]

[1]*Taipei Veteran General Hospital, Department of Internal Medicine, Taipei, Taiwan*
[2]*National Yang-Ming University, Taipei, Taiwan*

Background: The dysbiosis of gut microbiome and interaction with host immunity after Mycobacterium tuberculosis (MTB) infection are under-investigation.

Objectives: We hypothesized that the constitutional symptoms in patients infected with MTB were associated the dysbiosis in gut microbiota.

Methods: This was a prospective case-control study during 2014-2016 in a tertiary medical center including 25 active TB patients, 32 latent TB infection (LTBI) participants, and 23 healthy controls (HC). The gut microbiota was obtained from stools using V3-V5 16S rRNA gene sequencing method. Interleukin-1 beta (IL-1B), IL-4, IL-6, IL-10, CD3+, CD4+, CD8+ T cells, and interferon-gamma (IFN-γ) releasing assay (IGRA) were measured in peripheral blood.

Results: The ratio of *Firmicutes* to *Bacteroides* (F/B ratio) was an indicator for the dysbiosis of gut microbiome after MTB infection, which was highest in LTBI and lowest in HC or active TB ($p<0.05$). Presence of *Bifidobacteriaceae* or *Coriobacteriaceae* was negatively associated with clinical symptom ($p<0.05$). The leukocytosis (7032±387 cell/cum, $p<0.05$), increase in IL-6 (229.7±104μg/dL, $p<0.05$), and decrease in IL-4 (0.27μg/dL±0.1, $p<0.05$) were presented in active TB. The proportion of polymorphic neutrophil (PMN) in peripheral blood was positively related to the relative abundance of *Bacteroidetes* in LTBI and active TB ($R^2=0.23$, $p<0.05$). The F/B ratio was positively related to the detectable IL-1B in TB ($R^2=0.97$, $p<0.01$) and to the IL-4 in LTBI ($R^2=0.27$, $p<0.05$). In LTBI, the relative abundances of *Coriobacteriales* was positively related to the secretion of IFN-gamma against TB antigens more likely associated with of CD4+ T cell ($R^2=0.42$, $p<0.05$).

PT165 Handling a skin Microbiome project: Scientific watch associated to best practices for sampling, sequencing and analysis

Bernodat Nathalie[1], Audrey Gueniche[1], Nathalie Caron [1], Tarun Chopra[1], Cécile Clavaud[1], Florence Menard-Sczebara[2], Stéphanie Nouveau[2], Alban Ott[2], Olivier Perin[2], Tacheau Charlotte[2], Marie Thomas[2], Ahmad Khodr[2]

[1]L'Oréal Research and Innovation
[2]L'Oréal Research and Innovation

Background: Study design for skin microbiome research is multifaceted and integral to all downstream steps. Many published studies examined the biases introduced by the skin sampling methods (1) (2) and sample storage (3), controls and contamination sources (4), sequencing biases (5), and possible quantitation (6). The complexity of skin microbiome studies is nicely summarized in the title of the review from (7) "Performing Skin Microbiome Research: A Method to the Madness". Thus, standardization and validation of the protocols, bio-informatics pipelines and sequencing platforms are crucial and key parameters of a successful skin microbiome analysis.

Objectives: Set up of a standerized harmonized and validated piepline for **skin Microbiome** analysis and its application to evaluate the impact of a cosmetic skin care on Microbiome recovery after agression.

Methods: Review of the biases induced in skin Microbiome anlysis, SOPs for skin Microbiome samping.

Blinded ring test for skin Microbiome analysis platforms validation

Evaluation of the impact of the bioinformatic/biostatistical analysis

Application of the harmonized pipeline to evaluate skin Microbiome recovery in a clinical study

Results: Application of a standardized protocol from clinical design set up to biostatistical analysis permitted an accurate and subtitle evaluation of the impact of a cosmetic product on the skin Microbiome. Microbiome recovery after aggression of the skin was quicker with application of the skin care as shown by the evaluation of different parameters (bacterial load, alpha and Beta diversity).

PT166 A 16s rdna sequencing study towards the environment microbiome of powdered infant formula production sites

Yu Cao[1], Ankita Naithani[1], Shabarinath Srikumar[1], Séamus Fanning[1]

[1]*UCD-Centre for Food Safety, University College Dublin, Dublin, Ireland*

Background: Microbial safety is critically important for powdered infant formula (PIF) with neonates, with under developed immune systems, as the consumers. The prevalences of pathogens of importance to neonatal health, such as *Cronobacter sakazakii* and *Salmonella enterica*, are of great concern during PIF production.

Objectives: In this study, a 2-year environment monitoring was carried out in two PIF factories located in the Republic of Ireland. The environment microbiome in these two factories was studied to characterize the microbial baseline in the PIF production environment.

Methods: Environment sampling was carried out using sterile pre-moistened sponges and genomic DNA was extracted using a choloroform based method. 16S rDNA sequencing was carried out on a MiSeq platform and the data was analyzed using Mothur.

Results: Results highlighted a PIF factory environment core microbiome made up of 24 bacterial genera representing 5 phyla, with *Acinetobacter*, *Pseudomonas* and other unclassified Pseudomonadaceae families as the predominant genera. In different care areas of the factories, as hygiene standards increased, the microbial community structures over time became smaller and approached stability, and bacteria dominating the care area became less interfered by the external environment and more influenced by human and raw materials. Genera *Cronobacter* and *Salmonella* were observed in trace amounts in the PIF factory, and bacterial genera known to be persistent in stressed environment, such as *Bacillus*, *Streptococcus* and *Clostridium*, were likely to have higher abundances in dry environment-based care areas. Application of these methodologies advances the refinement of future food safety control.

PT167 Longitudinal homogenization of the human and built environment microbiome in a cohort of college roommates

Andrew Hoisington[1;2;3], Anukriti Sharma[4;5], Christopher Stamper[2], Juan Maestre[6], Kelly Stearns-Yoder[2;3;7], Teodor Postolache[2;3;8;9], Katherine Bates[3;10], Lisa Brenner[2;3;7;11], Jack Gilbert[5;12], Christopher Lowry[2;3;7;13;14;15]

[1]Air Force Institute of Technology, Department of Systems Engineering and Management
[2]Veterans Health Administration, Rocky Mountain Mental Illness Research Education and Clinical Center (MIRECC)
[3]Military and Veteran Microbiome Consortium for Research and Education (MVM-CoRE)
[4]University of Chicago, Department of Surgery
[5]Argonne National Laboratory, BioSciences
[6]University of Texas Austin, Department of Civil, Architectural and Environmental Engineering
[7]University of Colorado Anschutz Medical Campus, Department of Physical Medicine & Rehabilitation
[8]University of Maryland Baltimore, School of Medicine
[9]Veterans Health Administration, VISN 5 Mental Illness Research Education and Clinical Center (MIRECC)
[10]United States Air Force Academy, Department of Biology
[11]University of Colorado Anschutz Medical Campus, Departments of Psychiatry and Neurology
[12]University of San Diego, Department of Pediatrics
[13]University of Colorado Boulder, Department of Integrative Physiology
[14]University of Colorado Boulder, Center for Neuroscience
[15]University of Colorado Anschutz Medical Campus, Center for Neuroscience

Background: Humans spend much of their lives in the built environment, yet researchers are just starting to gather knowledge on the transmission of microbial communities between the occupants, from occupants to the built environment, and from the built environment to occupants.

Objectives: The overall goal of this study was to delineate the dynamic feedback between human and built environment microbiomes and factors that influence that response, through longitudinal characterization of cohabitation.

Methods: The study was conducted for five consecutive weeks and four additional weeks that included two break points in continuous sampling. Biweekly, human and built environment microbiome samples were collected at the United States Air Force Academy for 49 cadets roommates along with corresponding built environment samples and building characteristic measurements. DNA was sequenced on an Illumina MiSeq platform and analysis was conducted from 2,170 samples, after quality controls.

Results: The study observed an increase in skin microbial similarity of two individuals who start living together for the first time, and who are not genetically related or romantically involved. Cohabitation was significantly associated with increased skin microbiota similarity, but did not significantly influence the gut microbiota. Following a departure from the occupied space of several weeks, the skin microbiota, but not the gut microbiota, showed a significant reduction in similarity relative to the building. Through extensive metadata collection and analysis, it was observed that external

factors such as thermal comfort, stress (as measured in salivary cortisol), and urban or rural lifestyle during childhood influences the bacterial microbiome.

PT168 Metagenomic analysis of microbial communities associated with diseased potato tubers

Sanja Marković[1], Ivica Dimkić[2], Slaviša Stanković[2], Aleksandra Jelušić[3], Renata Iličić[4], Tatjana Popović[5]

[1]*Deka inžinjering, Novi Sad, Serbia*
[2]*Faculty of Biology, University of Belgrade, Belgrade, Serbia*
[3]*Institute for multidisciplinary research, Belgrade, Serbia*
[4]*Faculty of Agriculture, University of Novi Sad, Novi Sad, Serbia*
[5]*Institute for Plant Protection and Environment, Belgrade, Serbia*

Background: Pectolytic *Dickeya* and *Pectobacterium* species that cause blackleg, aerial stem rot and soft rot are commercially important pathogens in potato production. Blackleg symptom caused by these bacteria has been recorded in potato production field in Bačka region (Vojvodina, Serbia) in 2018.

Objectives: Metagenomic approach was used to reveal microbial communities colonizing the diseased potato tubers with the aim of better understanding of pathogens distribution.

Methods: Single colonies of potential pathogenic bacteria were isolated on nutrient agar and checked for pectolytic activity on potato slices. Ten selected bacterial isolates were identified to the species level after PCR amplification with *gapA* and *mdh* primers. The 16S rRNA sequences from diseased potato tubers samples were obtained using Next generation sequencing (NGS) Illumina platform. The obtained sequencing data was processed bioinformaticaly.

Results: Mixed infection of the diseased potato plants with *Dickeya dianthocola* and *Pectobacterium carotovorum* pv. *brasiliensis* was revealed after pathogen identification with *gapA* and *mdh* genes. All isolates caused soft rot symptoms on potato slices. Metagenomic analysis revealed that present bacteria belong to phylum Bacteroidetes (*Bacteroides*, *Empedobacter*, *Dysgonomonas*, *Myroides*, and ud-Porphyromonadaceae), Firmicutes (*Vagococcus*, *Lactococcus*, *Clostridium* XIVa, *Enterococcus*, and ud-Acidaminococcaceae) and Proteobacteria (*Acinetobacter*, ud-Pseudomonadaceae, ud-Enterobacteriaceae, *Wohlfahrtiimonas*, ud-Neisseriaceae, *Enterobacter*, *Providencia*, *Arcobacter*, and *Kerstersia*) as the most common representives. Genus *Pectobacterium* was present in 0.5%.

PT169 The Impact of Domestication on the Wheat Microbiota

Mohamed Amine Hassani[1], Ezgi Özkurt[1], Heike Seybold[1], Eva H. Stukenbrock[1]

[1]*Botanical Institute, Environmental Genomics, Kiel, Germany*

Background: Plants coexist with diverse microbial communities. The association between the plant and the microorganisms forms an ecological unit termed the holobiont. Plant-associated microbes can have beneficial, neutral or deleterious impact on their host fitness. It is therefore important to understand fundamental principles that govern the establishment of the plant microbiota toward better predicting dysbiosis-mediated diseases. It is well-documented that abiotic factors and host cues are key determinants of the plant microbiota. However, the evolutionary processes that mediate microbiota co-adaptation to the host are not well understood. Plant domestication provides a framework to glean data on the dynamic of the plant microbiota over short evolutionary times, identify selective pressures that shape the microbiota of domesticated plant species and formulate precise hypothesis regarding host-microbiota co-evolution.

Objectives: We aim over this project to i) study the role of domestication in altering the community structure and function of the wheat microbiota "bacterial and fungal communities", ii) establish a bacterial and fungal culture collections representative of the wheat leaf- and root-associated microbiota of wild or domesticated wheat and iii) decipher the interactions between the leaf microbiota of domesticated wheat and the fungal pathogen *Zymoseptoria tritici*.

Methods: To this end, we employed culture-independent microbial profiling to study the wheat leaf and root microbiota under healthy and disease states.

Results: Our preliminary results show that wild and domesticated wheat species harbor distinct microbial communities and that infection by *Z. tritici* significantly alters the leaf-associated bacterial communities of the domesticated wheat species.

PT170 The role of the microbiome in gill health of farmed Scottish salmon

Kelly Stewart[1], Umer Zeeshan Ijaz[2], Cindy Smith[2]

[1]*Rankine Building University of Glasgow, United Kingdom*
[2]*Rankine Building, United Kingdom*

Background: Scotland the EU's largest farmed Atlantic salmon producer, creating approximately 10,000 jobs and valued at £601 million in 2017 making salmon health vital for the industry. Gill disease is now a prominent issue for the salmon populations, leading to major mortalities. Recently, Complex Gill disease (CGD), thought to be multifactorial in nature with a wide range of clinical presentations, has emerged. Little is know about the cause or development of CGD.

Objectives: Here we aim to characterize and investigate the role of the gill microbiome in gill health and disease outbreaks in farmed Atlantic salmon throughout production cycles from freshwater hatcheries to final harvest from seawater cages.

Methods: To achieve this, we are tracking the gill microbiome of farmed salmon from four salmon farms located along the Scottish coast. This includes two Autumn seawater inputs (2018) and two Spring seawater inputs (2019) over a period of up to 18 months. Two farms are monitored intensely every two weeks, and two farms every 4 weeks. Characterisation of the gill microbiome will be preformed by 16S rRNA gene amplicon sequencing.

Results: To-date we have started sample collection, optimized DNA extraction and 16S rRNA primer region. We compared regions of the 16S rRNA gene to identify the optimal region to reduce host DNA contamination of our 16S libraries. This was significant for some regions, with up to 100% match of 16S rRNA primers with salmon DNA. We have therefore selected the V1 region to reduce host contamination of our amplicon libraries.

PT171 A gnotobiotic mouse model reveals microbial colonization dynamics in host intestine.

Jen Ji Hyun Yong[1], Mi Young Yoon[1], Sang Sun Yoon[1]

[1]*Yonsei University, Department of Microbiology and Immunology, , Seoul, Korea, Rep. of South*

Background: The commensal microorganisms inside host intestine, collectively termed gut microbiota, exert beneficial effects on the host. They can help defend against infections by bacterial pathogens. However, the protective role of gut microbiota against enteric infections is yet to be clearly defined. Furthermore, it still remains elusive how each microbial component of a gut microbiota colonizes the host intestine, ultimately formulating a stable symbiotic ecosystem.

Objectives: To address these important issues, we established a gnotobiotic mouse system with a defined consortium of commensal microbes.

Methods: Twelve microbial species that were frequently identified to be abundantly present among Korean population, which we have designated Major Gut Microbes among Koreans (MGMK), were transplanted into germ-free (GF) mice via oral gavage. Following transplantation, mouse feces were collected at intervals for 33 days and microbial populations present in each fecal sample were analyzed via 16S rRNA deep sequencing.

Results: Species belonging to the *Bacteroides* genus exhibited more competent colonization compared with other members of MGMK. Next, when gnotobiotic mice with MGMK and GF control mice were infected with *Vibrio cholerae*, an important human pathogen, MGMK-transplanted GF mice were more resistant to *V. cholerae* colonization. This result further validates that the presence of commensal microbes in the gut protect the host against colonization by pathogenic invaders.

PT172 Strain-Level Dynamics of the Lung Microbiome in Patients with Cystic Fibrosis

Marija Dmitrijeva[1;2], Christian Kahlert[3], Rounak Fiegelman[1;2], Rebekka Kleiner[4], Christian von Mering[1;2]

[1]*Institute of Molecular Life Sciences, University of Zurich, Zürich, Switzerland*
[2]*Swiss Institute of Bioinformatics, Zurich, Switzerland*
[3] *Infectious Diseases and Hospital Epidemiology, Cantonal Hospital St. Gallen, St. Gallen, Switzerland*
[4]*Pneumology and Sleep Medicine, Cantonal Hospital St. Gallen, St. Gallen, Switzerland*

Background: Cystic fibrosis (CF) is a life-threatening genetic disorder accompanied by chronic lung infections and respiratory complications that arise from the accumulation of viscous mucus in the airways. In the clinic, cultures of microbes isolated from the coughed-up lung sputum are used to decide on the CF management strategy. The approach, however, frequently misses non-typical microbes and provides limited strain-level resolution.

Objectives: In the study, we applied an unbiased sequencing approach to lung sputum samples in order to gain a more complete picture of the lung microbiome. We explored changes in the lung microbiome composition over time, particularly during exacerbations and antibiotic treatments. Moreover, we distinguished between evolution of internal strains and introduction of external strains from the environment.

Methods: We followed four CF patients displaying extreme clinical phenotypes over the course of two years. During each exacerbation or routine clinical visit, the lung sputum from these patients was taken for shotgun sequencing. Reads not mapping to the host were subjected to assembly, taxonomic profiling, strain typing, and variant analysis.

Results: Sequencing revealed the presence of several microbes missed by culture, particularly anaerobes. It allowed us to identify dominant strains of abundant microbes, and to correct mistakes in taxonomic assignment from culture. Finally, after analysing variation on the genome level, we describe multiple events such as a battle for dominance between two strains of Pseudomonas aeruginosa in the same patient. This study provides a first insight into the advantages of assessing the complete lung microbiome over time.

PT173 Analyzing holobiontic assosiations between host and microbiota in passerines

Jan Kubovčiak[1], Lucie Schmiedová[1], Tomáš Albrecht[1;2], Martin Těšický[1], Jean Francois Martin[3], Jakub Kreisinger[1;2]

[1]*Charles University in Prague, Faculty of Science, Department of Zoology, Prague, Czech Republic*
[2]*Institute of Vertebrate Biology, Academy of Sciences of the Czech Republic, Studenec, Czech Republic*
[3]*Centre de Biologie pour la Gestion des Populations, Montpellier, France*

Background: Increasing number of studies provide evidence of the fundamental influence of gut microbiota (GM) on the fitness and evolution of host species. In this context, multicellular organisms are considered as holobionts, i.e. naturally indivisible entities including the host organism and its associated microbiota.

Objectives: The aim of this study is to analyze GM of wildlife passerines in the Czech Republic and test whether the phylosymbiosis process takes place between the hosts and their gut microbiota. This phenomenon is a prerequisite to validate the Holobiont concept. Another aim is to identify individual components of GM, whose composition is host-species specific and is potentially shaped by coevolution process.

Methods: Using the Illumina MiSeq platform, 16s rRNA bacterial gene amplicons from faecal samples of 486 individuals representing 57 species from the Czech Republic were sequenced. The evidence of phylosymbiosis was performed by methods based on the GM composition variance between individuals with respect to their phylogeny. To determine this phenomenon for individual GM components, haplotypes clustering on a sequence similarity threshold of 95 % were analyzed similarly.

Results: Ongoing phylosymbiosis between GM and the host group was detected, although the effect is rather mild. Furthermore, individual haplotypes, whose composition exhibits a high degree of dependence on the taxonomic relationships of the host species, have been identified. Among these we found for example group of bacteria assigned to genera *Candidatus Arthromitus*, known for facilitating host immune system development in vertebrates.

PT174 Combining metaomics approaches to understand the process of human gut microbiota development

Alejandra Rey Mariño[1], Susana Ruiz Ruiz[1], Nuria Jiménez Hernández[1], Javier Pons[1], Alejandro Artacho[1], Pilar Francino[1;2]

[1]Centro Superior de Investigación en Salud Pública, Genómica y Salud, València, Spain
[2]CIBER, Epidemiología y Salud Pública (CIBERESP), Madrid, Spain

Background: The intestinal microbiota is deployed along the development of the individual and its composition and functions differ depending on age. Knowing about the changes that occur in the gut microbiota throughout life can help the diagnosis, treatment and prevention of diseases related to metabolic and inmune alterations. The combination of the different high-throughput metaomics is key to understand the process of intestinal microbiota development and at what level and to what extent these changes occur.

Objectives: We intend to understand the process of intestinal microbiota development by analyzing compositional and functional changes in a prospective cohort of 12 toddlers, 13 adolescents and 35 adults in order to establish at what age the microbiota reaches typical adult characteristics.

Methods: We performed metagenomic and metatranscriptomic analysis to obtain measurements of microbial species, gene and gene transcript composition of the gut microbiota of toddlers, adolescents and adults for a year and a half.

Results: We observe that the important differences that remain between the gut microbiota of infants and that of adults progressively subside during childhood and adolescence. At the taxonomic level, one main difference between toddlers, on one hand, and adolescents and adults, on the other, is the elevated abundance of *Bifidobacterium*. Our results show a directional change toward the taxonomic composition, functional composition and gene expression patterns of the adult microbiome. However, in adolescents, some gene abundances and expression levels still are found to differ from those of adults.

PT175 Temporal variation in composition and function of the intestinal microbiota from Atlantic salmon treated with Bacitracin/Neomycin

Rodrigo Vargas[1;2], Joselin Rojas[1;2], Natalia Valdes[1;2], Alex Gonzalez[3], Mario Tello[1;2]

[1]*Facultad de Química y Biología, Biologia, Santiago, Chile*
[2]*Centro de Biotecnología Acuicola, Santiago, Chile*
[3]*Laboratorio de Microbiología Ambiental, Ciencias Biológicas y Biodiversidad, Osorno, Chile*

Background: Chilean salmoniculture use a large amount of antibiotics against bacterial infectious. The effect of antibiotics on the microbiome and its functional properties has been scarcely studied in the Atlantic Salmon.

Objectives: The goal of this study is evaluate the taxonomic transitions of the microbiome in the intestine of salmons treated with non-absorbable antibiotics, and infer the changes in the functional properties of the microbiome.

Methods: Pre-smolt fish divided into groups were used: control (C), treated with the antibiotics Bacitracine and Neomycin for 14 days (Ab) and recovery group which was left for 14 days without antibiotic treatment (R). Massive sequencing of the 16S rRNA V3-V4 region was done from fecal and intestine samples. The metagenomic analysis, taxonomic classification and metabolic prediction were made with the QIIME and PICRUSt programs.

Results: At Filum the group C in feces and intestine dominates Proteobacteria, in the group (AB) in feces dominates Firmicutes and in intestine Proteobacteria; in the group R Firmicutes dominates feces and intestine. At the level of genus, group C is dominated by Flavobacterium in feces and Shewanella in intestine; in the group AB Lactococcus and Lactobacillus dominate in feces and Jhantinobacterium the intestine; Group R is dominated by Lactococcus and Streptococcus in feces and Lactococcus in intestine. The functional inferential shows a greater representation of all the modules evaluated in feces and intestine of group AB. These results show that antibiotics changes the predominance of certain groups and increase the diversity of metabolic pathways present in the microbiome.

PT176 Heat-killed lactobacilli impact microbiota composition and host behaviour

Alicja Warda[1], Cara Hueston[1], Adam Clooney[1], Colin Hill[1;2]

[1]*APC Microbiome Ireland, Cork, Ireland*
[2]*University College Cork, School of Microbiology, Cork, Ireland*

Background: Live microorganisms in particular probiotics are known to, when administered in adequate amounts, confer a health benefit on the host. Hosts' benefits include promotion of a healthy digestive tract, immune system, and gut–brain axis. However, limited data exist on the impact of dead bacteria or heat inactivated fermentates on host health.

Objectives: The objective of this study was to evaluate the effect of a heat-killed fermentate containing two inactivated *Lactobacillus* strains, on microbiota composition, host health and behaviour.

Methods: We used murine models relevant to behaviour parameters (stress, depression, anxiety and cognition), infection and inflammation. The effect on human microbiota was studied using anaerobic batch fermentations inoculated with human standardised faecal slurry. Analysis included 16S rRNA analysis of microbiota, virome analysis, SCFA, and when appropriate inflammation markers and/or behaviour with and without fermentate supplementation of chow and/or fermentation vessels.

Results: Heat-killed fermentate of lactobacilli had an effect on number of measurements. The supplementation led to subtle but significant changes in both human and murine microbiota, with less abundant taxa being most affected in the later case. While prolonged consumption of fermentate had no adverse effect on murine health, fermentate-fed animals demonstrated increased sociability and lower corticosterone baseline levels suggesting a 'calming' effect of fermentate.

The behavioural, biochemical and microbiological tests provide new light on the effects of inactivated bacteria and their metabolites on the murine and human microbiota, inflammation of infected animals and healthy mice behaviour.

PT177 Influence of microwave sanitation on the metagenome of used kitchen sponges

Susanne Jacksch[1], Jyothi Thota[1], Markus Egert[1]

[1]*Institute of Precision Medicine, Furtwangen University, Villingen-Schwenningen, Germany*

Background: Being able to collect and spread microorganisms, domestic kitchen sponges are a source for microbial contaminations of surfaces and food [1]. Sanitation methods, such as microwave treatment, may cause a short-term reduction of the microbial load [2], however, long-term effects on microbiome structure and function are totally unknown.

Objectives: We investigated the effect of regular microwave sanitation on the microbiome composition of kitchen sponges by means of shotgun sequencing.

Methods: To have a controlled experimental setup, newly bought polyurethane kitchen sponges were issued to the 20 participants of this study. Each sponge was used under normal domestic kitchen conditions for ~ 4 weeks. Ten participants were instructed to clean their sponge regularly by standardized microwaving, while the remaining ten sponges were used without any special sanitation. After DNA extraction and Illumina-MiSeq-based shotgun sequencing, all data were uploaded to the MG-RAST platform for taxonomic annotation

Results and Discussion: Preliminary data analysis revealed that 97.0% of all sequences belonged to the domain of bacteria. However, viral (2.7%), eukaryotic (0.2%) and archaeal (0,006%) sequences were also found. At bacterial phylum level, *Proteobacteria* (85.6%), *Bacteroidetes* (7.4%) and *Actinobacteria* (3.6%) were relatively most abundant, while *Acinetobacter*, *Enhydrobacter*, *Agrobacterium* and *Pseudomonas* were the dominant genera. Diversity analyses showed a trend towards lower microbial diversity in sponge samples that had been microwaved regularly. The effect on the pathogenic potential of the bacterial microbiota [1] is under investigation.

[1] Cardinale et al. (2017) Sci Rep 7(1): 5791, [2] Sharma et al. (2009) Food Control 20(3): 310–313

PT178 Gut Microbiota shifts in pigs related to Antibiotics and Phytogenics

Verity Ann Sattler[1], Paul D. Cotter[2], Fiona Crispie[2], Birgit Antlinger[1], Iris Schantl[1], Veronika Nagl[1], Viviana Klose[1], Nicole Reisinger[1]

[1]BIOMIN Holding GmbH, BIOMIN Research Center, Tulln an der Donau, Austria
[2]Teagasc Food Research Centre and APC Microbiome Ireland, Moorepark, Fermoy, Ireland

Background: The gut microbiota plays a major role in the maintanence of animal health and optimal growth. Alternatives to antibiotics for food-producing animals are of interest to help improve animal performance and reduce the spread of antibiotic resistance genes.

Objectives: The aim of this study was to investigate differences in the microbiota diversity and composition, between pigs receiving either an antibiotic or phytogenic feed additive.

Methods: A total of 24 piglets were divided to: antibiotic group, receiving a standard diet with 10g Oxytetracyclin (OTC)/100kg BW/day for 7 days; phytogenic group, receiving a standard diet with Digestarom DC XCel® 150g/t; and control group receiving the standard diet only, until day 21. Feces samples were collected from each pig at day 0, day 8, and day 21 of the study. The gut microbiota was characterized based on 16S rRNA gene amplicon sequencing using the MiSeq-Illumina technology.

Results: Faecal microbiota composition changed over time and showed differences between animals related to diet. At day8, a reduced bacterial diversity (Chao1) and richness was observed in the OTC group compared to the control and phytogenic group. Bacteroides and Desulfovibrio were enriched in the antibiotic and decreased in the phytogenic group, compared to control. Pirellulaceae and Fibrobacteriaceae were decreased in the antibiotic compared to control group. Multivariate statistics (Bray-Curtis) showed differences in microbiota communies related to diet at day8, but not at day0 and 21. Microbiota analysis of three sick animals, medicated with fluoroquinolone, will also be performed and compared to healthy, unmedicated animals.

PT179 Does synanthropy take guts? Comparison of bacteriome and mycobiome of two mouse species with different ecologies

Barbora Bendová[1;1;2], Jakub Kreisinger[1;2], Lucie Schmiedová[1;2], Dagmar Čížková[2], Jaroslav Piálek[2], Ľudovít Ďureje[2], Jean-Francois Martin[3]

[1]*Faculty of Science, Charles University, Prague, Czech Republic, Zoology, Prague, Czech Republic*
[2]*The Czech Academy of Sciences, Institute of Vertebrate Biology, Brno, Czech Republic*
[3]*Montpellier-SupAgro, UMR CBGP, Montferrier-sur-Lez, France*

Background: The vertebrate gastrointestinal tract is colonized by microbiota, which has major effect on host's health, physiology and phenotype. Gut microbiota composition changes dramatically after the introduction of free-living individuals to captivity. However, only little is known about changes in gut microbiota associated with adaptation to synanthropic lifestyle in commensal species compared to their non-commensal counterparts.

Objectives: Here, we aimed to compare the taxonomic composition and diversity of bacterial and fungal communities across three gut sections in synanthropic house mouse (*Mus musculus*) and its non-synanthropic counterpart, the mound-building mouse (*Mus spicilegus*).

Methods: Individuals of both species were caught in two neighbouring localities in eastern Slovakia and from these three different gut section samples were obtained. The microbial composition and diversity was analysed using Illumina sequencing of bacterial 16S rRNA and fungal internal transcribed spacer 2 (ITS2) region amplicons.

Results: We found higher bacterial diversity in *M. spicilegus* and detected 11 bacterial operational taxonomic units with significantly different proportions in the compared mice. An example genus *Oscillospira* which was more abundant in *M. spicilegus* typically increases in lean or outdoor pasturing animals. ITS2 based barcoding revealed low diversity and high uniformity of gut fungi in the two species with major dominance of genus *Kazachstania*. Despite the observed differences in gut bacteria in the two species which can be associated to the tightness of their association to humans, the previously reported changes in microbiota due to changes from commensalism to captivity seem to cause larger microbiota shifts.

PT180 Topographical diversity of the upper respiratory tract microbiome in chronic rhinosinusitis patients

Ilke De Boeck[1], Stijn Wittouck[1], Katleen Martens[1;2], Jos Claes[3], Mark Jorissen[4], Brecht Steelant[2], Marianne van den Broek[1], Sven Seys[2], Peter Hellings[4;5], Olivier Vanderveken[3;6], Sarah Lebeer[1]

[1]University of Antwerp, Department of Bioscience Engineering, Antwerpen, Belgium
[2]KU Leuven, Department of Microbiology and Immunology, Leuven, Belgium
[3]Antwerp University Hospital, ENT, Head and Neck Surgery, Edegem, Belgium
[4]University Hospitals Leuven, Clinical Department of Otorhinolaryngology, Head and Neck Surgery, Leuven, Belgium
[2]KU Leuven, Department of Microbiology and Immunology, Leuven, Belgium
[6]University of Antwerp, Faculty of Medicine and Health sciences, Translational Neurosciences, Wilrijk,

Background: It is generally believed that the microbiome plays a role in the pathophysiology of chronic rhinosinusitis (CRS), though the exact contribution in disease development and severity remains unclear. Furthermore, much remains to be discovered about the occurrence of potential pathobionts in different niches of the upper respiratory tract (URT).

Objectives: In this study, we aimed to investigate the topographical differences and continuity of four different URT niches of CRS patients to gain information on the occurrence of potential pathobionts and beneficial microorganisms in specific locations of the URT. Furthermore, we aimed to compare the URT microbiome of these CRS patients with healthy individuals.

Methods: Samples from the anterior nares, nasopharynx, maxillary and ethmoid sinus were collected from 225 CRS patients, and from anterior nares and nasopharynx from 100 controls. Microbial communities were analyzed by *16S rRNA* V4 Illumina sequencing, followed by analysis at the amplicon sequence variant (ASV) level.

Results: Our data indicate a rather strong continuity for the microbiome in the different URT niches in CRS patients, with the anterior nares most similar to the sinus microbiome. This is striking since the anterior nares can be seen as an open ecosystem, while the nasopharynx and sinuses are more closed ecosystems. Bacterial alpha-diversity was impaired in CRS without nasal polyps compared to controls, but not in CRS with nasal polyps. *Dolosigranulum pigrum* was more associated with healthy controls, while *Corynebacterium tuberculostearicum*, *Haemophilus influenzae/aegyptius* and *Staphylococcus* taxa were found as potential CRS pathobionts.

PT181 Intestinal colonization with carbapenemase or ESBL producing Enterobacteriaceae in continuing-care facilities in North of Portugal

Rita Sofia Conde Lopes[1], Daniela Gonçalves[2], Helena Maria Neto Ferreira[1]

[1]*University of Porto - Faculty of Pharmacy, Porto, Portugal*
[2]*ISAVE - Higher Institute of Health*

Background: Population aging creates a greater need for medium/long term care facilities, as those of the Integrated-Continuing-Care-Units (ICCU). Multidrug-resistant bacteria (MDR), especially Gram-negative, such as *Escherichia coli* (*E. coli*) and *Klebsiella pneumoniae* (*K. pneumoniae*) emerge as important threats.

Objectives: Detection and characterization of multi-resistant Gram-negative bacilli, as intestinal colonizers, in ICCU patients, particularly extended-spectrum-β-lactamase (ESBL) and carbapenemase-producing Enterobacteriaceae.

Methods: Forty fecal samples of patients from 3 ICCU of the North of Portugal, districts of Bragança (23), Porto (11) and Braga (6), were analyzed. β-lactam antibiotic susceptibility test was performed according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) specifications. ESBL producers were confirmed by addition of clavulanic acid to cefotaxime disks, in the diffusion method. Carbapenemase-producers were detected by Blue-carba test, Modified Hogde test and Carbapenem Inactivation Method. ESBL and carbapenemase genes were characterized by polymerase chain reaction (PCR) and sequencing for KPC producer.

Results: Thirty-one relevant intestinal isolates were identified: 30 ESBL and 1 carbapenemase producers (*K. pneumoniae*). Molecular characterization showed one isolate producing KPC-2 type and others CTX-M-group 1, particularly CTX-M-15. Some patients showed co-colonization. In patients of ICCU from Portuguese health-care setting, intestinal colonization by ESBL producers is already a reality and carbapenemases have shown a growing incidence. These units may function as effective vehicles in the spread of MDR bacteria and resistance genes. Intestinal colonization and spread of bacteria with acquired multidrug-resistance is a reality, and is a major priority that population and health units take serious infection control measures and use antibiotics rationally.

PT182 Host Susceptibility to Vibrio cholerae Infection is Determined by Intestinal Abundance of Bacteroides vulgatus

Jen Ji Hyun Yong[1], Jinsun You[1], Mi Young Yoon[1], Sang Sun Yoon[1]

[1]Yonsei University College of Medicine, Dept. of Micrbiology and Immunology, Seoul, Korea, Rep. of South

Background: Perturbations of the gut microbiota induced by events such as antibiotic treatment can significantly suppress the protective capacity of the gut microbiome, termed 'colonization resistance'. Under such circumstances, host becomes more susceptible to infections by various pathogens such as *Shigella flexneri* and *Clostridium difficile. Vibrio cholerae* is another important human pathogenic bacterium that binds to the human intestinal epithelium and mediates a severe watery diarrhea through secretion of cholera toxin (CTX). However, how antibiotic-induced perturbations of the gut microbiota influences the host's susceptibility to *V. cholerae* infection remains unexplored.

Objectives: Our main objective in this study was to illustrate the effects of different antibiotics on the indigenous gut microbiome, and how such changes relate to the host's resistance to *V. cholerae* infection.

Methods: *In vivo* experiments utilised C57BL/6 Specific Pathogen Free (SPF) and Germ Free (GF) mice. SPF mice were administered clindamycin (CL) and infected with *V. cholerae* by oral gavage. GF mice were mono-associated with *Bacteroides vulgatus* isolated from the feces of SPF mice, and infected with *V. cholerae.* Fecal pellets were collected for community profiling by 16S rRNA gene sequencing, and used to extract bacterial genomic DNA, which was subsequently used for real-time PCR.

Results: CL treatment completely eradicates *B. vulgatus* from the mouse intestines. Both in SPF and GF mice, absence of *B. vulgatus* positively correlated with intestinal *V. cholerae* load. Our results indicate that the presence of *B. vulgatus* is a critical determinant for host resistance against *V. cholerae* infection.

PT183 Gut Microbiota of Large Breed Dogs Using Miseq NGS Technique: A Prospective Follow-up Study from Lactating Stage to Puppy Stage

Jae-Uk An[1], Seung-Hyun Mun[1], Kyungwan Park[2], Junhyung Kim[1], Seongbeom Cho[1]

[1]BK21 PLUS Program for Creative Veterinary Science Research, Research Institute for Veterinary Science and College of Veterinary Medicine, Seoul National University, Seoul, Korea, Rep. of South
[2]Military working dog training center, Republic of Korea Army, Korea, Rep. of South

Background: Gut microbiota is a concept that includes all the microorganisms present in the GI tract of the host, including bacteria, virus, fungi, and protozoa. They are known to play a beneficial role in helping digestion and absorption of nutrients through a symbiotic relationship with the host, or by preventing the entry of exogenous pathogens into the host.

Objectives: This study aims to compare the bacterial gut microbiota of two large breed dogs raised in the same environment.

Methods: Fecal samples were collected from offspring of two different breed dogs (10 and 7 dogs, respectively). Each group was sampled seven times considering changes in feed including lactation stage until 4 weeks, both lactation and weaning from 4 to 6 weeks, weaning from 6 to 11 weeks, and post-weaning from 11 to 28 weeks. Metagenome was extracted from each fecal sample, and microbiota composition analysis was performed using Miseq NGS technique.

Results: Four phyla were over 96% of the total taxonomic composition. Bacteroidetes (24.21 to 52.05%), Firmicutes (30.36 to 57.95%), Fusobacteria (6.81 to 18.46%), and Proteobacteria (4.86 to 17.46%), respectively. In the case of 6, 8, 11, and 14-weeks samples, we found the different proportion of 4 major phyla (Kruskal-Wallis test, $P<0.05$). On the contrary, 21 and 28-weeks samples were no difference in phylum between dog breeds. Despite living in the same breeding environment and eating the same diet, gut microbial compositions are not be clearly matched between different breeds.

PT184 Flying to Mars, the salivary microbiota under isolated conditions

Giovanni Bacci[1], Edoardo Cipriani[1], Giovanni Emiliani[1], Giovanna Bianconi[2], Francesco Canganella[2], Alessio Mengoni[1], Renato Fani[1]

[1]*Università degli Studi di Firenze - Dipartimento di Biologia, Sesto Fiorentino, Italy*
[2]*Università Della Tuscia, Department of Biological, Agricultural and Forestry Sciences, Viterbo, Italy*

Background: The human oral cavity contains complex bacterial communities. The salivary microbiome has gained attention for its association with many human diseases. Unfortunately, it has been shown to be highly influenced by the surrounding environment more than by any other subject-specific factor (e.g. host genetics, age, sex …), making the interpretation of its composition in relation to subject status (e.g. health) problematic.

Objectives: Understand how sharing the same environment for a prolonged period of isolation may affect the composition of the salivary microbiome in healthy subjects.

Methods: Salivary samples were collected during the Mars500 mission (520 days) directly by the 6 crew members. Samples were then subjected to targeted metagenomic on 16S rRNA gene. Sequences were clustered using the UPARSE pipeline and used for downstream analyses.

Results: The alpha-diversity of subjects was similar during the whole mission, whereas the beta-diversity showed a decreasing trend along isolation period, allowing to measure the effect of co-housing on subject-specific salivary microbiota. A significant effect of time, diet, and subjects on the overall bacterial community composition was detected. The decreasing trend in subject-by-subject diversity was observed during the whole period of isolation and even during the follow-up (250 days after the end of the isolation). This trend was due to massive change of salivary microbiota, accounting for more than 40% of identified taxa. This work paves the way for quantitatively assessing, in a controlled experiment, the effect of co-housing on the salivary microbiota.

PT185 Gut microbiota of women with gestational diabetes mellitus and foetal macrosomia

Katja Molan[1], Zdravko Podlesek[1], Drazenka Pongrac Barlovic[2], Marjanca Starčič Erjavec[1], Jerneja Ambrožič Avguštin[1], Darja Žgur Bertok[1]

[1]University of Ljubljana, Biotechnical faculty, Department of Biology, Ljubljana,
[2]University medical centre Ljubljana, Clinical Department of Endocrinology, Diabetes and Metabolic Diseases

Background: Gut microbiota is crucial for human health. Dysbiosis has been associated with various diseases, among them also gestational diabetes (GDM). GDM is a risk factor for foetal macrosomia, child's obesity and diabetes mellitus of the mother and the child.

Objectives: Microbiota of women with gestational diabetes mellitus (GDM-women) in conjunction with foetal macrosomia has not been studied yet. We hypothesized that differences in gut microbiota exist in GDM-women who delivered macrosomic babies in comparison with GDM-women who delivered normal weight babies.

Methods: GDM-women were extensively phenotyped in the second trimester of pregnancy. 16S metagenomic analysis of faecal gut microbiota by NGS was performed in the second and third trimester.

Results: Among all recruited GDM-women, 20 were selected (age 33±4 years, body mass index 25±5 kg/m^2, weight gain during pregnancy 10±5 kg, glycated haemoglobin 5.0±0.3%) for 16S sequencing of faecal metagenomes. Five of them delivered a macrosomic baby. 16S sequencing revealed that GDM-women with macrosomic babies had a lower abundance of *Firmicutes* (*p=0.028*) driven by a lower abundance of *Clostridia* (*p=0.028*) in the 2nd trimester. At the bacterial family level, several significant differences were also observed in both trimesters (e.g. lower *Alcaligenaceae, Moraxellaceae, Xanthomonadaceae* in GDM-women with macrosomic babies), indicating persistent changes associated with macrosomia. Regarding the phenotype, these women were older (37±3 vs. 31±4 years, *p=0.008*), had higher serum triglycerides (2.6±0.2 vs. 1.9±0.5 mmol/l, *p=0.002*), while glycemic control and weight gain were comparable in both groups.

PT186 Comparative metagenomics of microbial communities inhabiting the phyllosphere of the diseased and healthy oilseed rape

Aleksandra Jelušić[1], Ivica Dimkić[2], Tanja Berić[2], Petar Mitrović[3], Sanja Marković[4], Slaviša Stanković[2], Tatjana Popović[5]

[1]*Institute for multidisciplinary research, Belgrade, Serbia*
[2]*Faculty of Biology, University of Belgrade, Belgrade, Serbia*
[3]*Institute of Field and Vegetable crops, Novi Sad, Serbia*
[4]*Deka inžinjering, Belgrade, Serbia*
[5]*Institute for Plant Protection and Environment, Belgrade, Serbia*

Background: A wide variety of different saprophytic and pathogenic bactera, both living epiphytic or endophytic, colonize physically and nutritionally different microhabitats on plants phyllosphere. *Xanthomonas campestris* pv. *campestris* (Xcc) is phytopathogenic bacteria causing bacterial blight symptom on oilseed rape (*Brassica napus*) and black rot on other cultivated *Brassica oleaceae* vegetables.

Objectives: Metagenomic approach was used to reveal differences in microbial communities colonizing the phyllosphere of four different winter oilseed rape varieties with expressed bacterial blight symptoms and one healthy variety, all collected from five different localities in Vojvodina (Serbia) in 2018.

Methods: Next generation sequencing (NGS) Illumina MiSeq platform was used to obtain 16S rRNA sequences from five tested samples, and to gain insight into their alpha and beta diversity. Comparative taxonomic analysis of the obtained sequencing metadata was processed bioinformaticaly.

Results: Different percetages of bacterial species belonging to three bacterial phyla-Proteobacteria, Firmicutes, and Actinobacteria were dominant in phyllosphere of all five oilseed rape varietes. Bacteria from the phylum Proteobacteria (66-91%) were dominant in diseased varieties, unlike healthy variety where only 38% belonged to this phylum. Genus *Xanthomonas* (40-74%) was the most abundant in diseased plants phyllosphere. Only 0.2% of *Xanthomonas* representatives were present in healthy plants, where order Enterobacteriales (*Pantoea*, *Buchnera*, and ud-Enterobacteriaceae) were the most abundant. The genus *Exiguobacterium* was the most abundant in healthy variety (47%). Bacteria from phylum Acintinobacteria were least represented, with less then 1% in diseased and up to 12% (ud-Micrococcaceae, *Kocuria*, *Arthrobacter*, and ud-Microbacteriaceae) in healthy oilseed rape variety.

PT187 Fecal carriage of multi-drug-resistant bacteria in hospital ward - a silent threat

Diana Marques[1], Daniela Gonçalves[2], Helena Maria Neto Ferreira[1]

[1]*University of Porto - Faculty of Pharmacy*
[2]*ISAVE - Higher Institute of Health*

Background: Admission of colonized patients with multi-drug-resistant (MDR) bacteria represents an added risk of antibiotic-resistance dissemination, outbreak installation and clinical complications in susceptible patients.

Objectives: We aimed to characterize MDR isolates colonizing patients in a neurology hospital ward.

Methods: Twenty-eight faecal samples were collected. Isolates were selected in MacConkey agar and MacConkey agar supplemented with cefotaxime, ciprofloxacin and meropenem (2 µg/mL). Colonies in antibiotic supplemented media were selected for further studies, according to the EUCAST. Presumptive identification was achieved by CHROMagar™ Orientation and confirmation by ID32GN. Extended-spectrum beta-lactamase (ESBL), carbapenemase-producing isolates and *Escherichia coli* (*E. coli*) phylogenetic group plus quinolone resistance were studied by PCR. Carbapenemase-producing isolates were subjected to sequencing of the codifying gene.

Results: Twenty-two of 28 samples were selected for study. We found isolates producing ESBL, carbapenemases (KPC-2), plasmidic AmpC β-lactamases and ciprofloxacin resistance. We found ESBL producers harbouring $bla_{\text{CTX-M-group1}}$, $bla_{\text{CTX-M-group9}}$, $bla_{\text{CTX-M-15}}$, bla_{TEM}, bla_{OXA} and bla_{SHV}. We found *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *E. coli*, *Enterobacter cloacae* and *Proteus* spp resistant to antibiotics. Some were co-colonizing the patient. Most *E. coli* belonged to B2 and D phylogenetic-groups. Both B2 were O25b-ST131 clonal group.

Intestinal carriage of MDR bacteria represents a hidden reservoir and risk for dispersion of these threats. Results show the importance of patient microbiota analysis during hospitalization in order to avoid outbreaks and clinical complications. Detection of intestinal colonization with resistant bacteria is relevant in terms of infection control to prevent intra-hospital dissemination and to avoid community spread after patient discharge.

PT188 Escherichia coli high-risk clone - influence of the elder population in dissemination

Daniela Gonçalves[2], Pedro Cecílio[1], Helena Maria Neto Ferreira[1]

[1]*University of Porto, Portugal*
[2]*ISAVE - Higher Institute of Health, Amares, Portugal*

Background: Pandemic multi-resistant and virulent *Escherichia coli* (*E. coli*) O25b-ST131 has been repeatedly associated with the health-care network. Nursing-homes (NH) and long-term-care-facilities (LTCF), usually receive patients discharged from the acute-care setting, potentially colonized with CTX-M-15 producers.

Objectives: Our main goal is to describe the successful installation of CTX-M-15-producing-O25b-ST131 clonal-group *E. coli* in the hospital setting and extra-hospital institutions (7NH and 3LTCF) in the same geographical area.

Methods: Susceptibility was accessed by disc diffusion methods according to the CLSI, and bacterial identification of intestinal colonization isolates was achieved by API20E and ID32GN and by Vitek2-system in clinical isolates. ESBL-producers were confirmed by E-test and the double-disk-synergy-test. PCR was performed for detection of TEM/OXA/SHV/CTX-M-group1/CTX-M-15 genes, phylogenetic-groups, O25b-ST131-clonal-group, genes coding for resistance to non-β-lactam-antibiotics and twenty-two virulence-factors. Sequencing was performed using group-specific primers for β-lactamase-genes. *E. coli* isolates relationship was addressed by pulsed-field-gel-electrophoresis (PFGE).

Results: Fifty-eight CTX-M-15-producing-*E. coli*-O25b-ST131 isolates were detected and clustered in nine PFGE-profiles (≥80%-homology). Each PFGE type contains clinical isolates from different biological products, and intestinal colonization isolates. These isolates showed also extended-resistance-phenotype to non-β-lactam-antibiotics and a virulent profile. Clonality relationships of CTX-M-producing-*E. coli*-O25b-ST131, alert to the circulation of patients between different types of care, hospital and extra-hospital institutions, favoring the silent and complex dissemination of these multi-resistant bacteria in elder-care network. Intestinal colonization is a silent form of dissemination of CTX-M-15-producing-*E. coli* and resistance genes, promoted by patient circulation through the healthcare network, and an important risk of input of these high-risk clones in hospital environment.

PT189 Development of a methodology for the restoration of the intestinal mucus layer after antibiotic therapy

Madiyar Nurgaziyev[1], Ayaulym Nurgozhina[1], Samat Kozhakhmetov[1], Almagul Kushugulova[1], Kairat Rysbekov[2], Rashida Satybayeva[2]

[1]National Laboratory Astana, Laboratory human microbiome and longevity, Astana, Kazakhstan
[2]City Children's Hospital 2, Astana, Kazakhstan

Background: Currently, there is a sufficient number of studies demonstrating the effectiveness of probiotic strains in correcting the adverse effects of antibacterial drugs, in particular antibiotic-associated diarrhea. However, to date, no pathogenetic mechanisms of probiotics influence on the state of the mucin layer have been established and there is no convincing evidence base on the mechanisms of their interaction with human microbiocenosis.

Objectives: The objective of this research is to establish the pathogenetic features of destabilization of the microbiocenosis of the gastrointestinal tract, changes in the state of intestinal mucus layer during therapy with antibacterial drugs and develop methodologies for restoring the system of microbiocenosis and mucus layer of the intestine after antibiotic therapy.

Methods: 12 children aged 4 - 5 with inflammatory diseases of the respiratory system and receiving therapy with antibacterial drugs of cephalosporin class were selected as participants for the study. The children were divided into 2 groups. Group 1 received probiotic in parallel to the main treatment. Group 2 received only basic treatment. Total DNA was isolated from fecal samples, amplified and sequenced to determine faecal microbiota composition. Immunological status studies were carried out in accordance with the Protocols for MAGPIX Multiplexing Instrument.

Results: The results of microbiome analysis in the dynamics of antibiotic therapy demonstrated significant changes in the Shannon index, as well as structural changes in the intestinal microbiota at the level of genera and species. Analysis of local immunity showed distinct changes in pro-inflammatory and anti-inflammatory cytokines, and also significantly increased immunoregulatory index.

PT190 Dysbiosis of the gastric microbiota is related to gastric cancer risk in Colombian populations

Alvaro Pazos Moncayo[1;2], Juan Camilo Caguazango[3]

[1]*Universidad de Nariño, Department of Biology, Nariño, Pasto, Colombia*
[2]*Public Health Research Group, Universidad de Nariño, Nariño, Pasto, Colombia*
[3]*Public Health Research Group, Center for Health Studies, Universidad de Nariño, Nariño, Pasto, Colombia*

Background: The dysbiosis of the gastric microbiota has been proposed as a risk factor for gastric cancer. Our hypothesis postulates that the changes-dysbiosis of the accompanying microbiota to *H. pylori* is related to the population risk to gastric cancer and the type of gastric lesion.

Objectives: We evaluate the changes-dysbiosis in the profile of the gastric microbiota associated with *H. pylori* in two Colombian populations with contrast in the risk of gastric cancer.

Methods: 409 adults of Tuquerres-Colombia: high risk of gastric cancer and Tumaco-Colombia: low risk of gastric cancer were included. Gastric lesions were classified by the Sydney system. The gastric microbiota was determined by sequencing the *16S rRNA* gene (V3-V4) by illumine-Miseq 2x300 pb. The OTUs were identified by BLASTn and RDPII. The microbial composition was compared according to population at risk of gastric cancer, *H. pylori* status and type of gastric lesion, by Permanova test and alpha-beta diversity analysis.

Results: Significant differences were found in the gastric microbiota profiles associated with *H. pylor*i, according to population at risk of gastric cancer *(p* < 0.0001). Statistical differences were observed in the composition of the accompanying microbiota to *H. pylor*i, between patients with non-atrophic gastritis and patients with intestinal metaplasia *(p* ≤ 0.05). The gastric microbiota composition associated with *H. pylori* is related to the population risk to gastric cancer and to the type of gastric lesion. The differential dysbiosis of the gastric microbiota could modulate the contrasting risk to gastric cancer between the two populations of Colombia.

PT191 Possible links of daily food products to the microbiome of a human

Ayaulym Nurgozhina[1], Almagul Kushugulova[1], Samat Kozhakhmetov[1], Madiyar Nurgaziyev[1], Nazar Seydalin[2], Roza Bakenova[2]

[1]Kazakhstan, Human microbiome and longevity, Astana, Kazakhstan
[2]Medical Centre Hospital of president's affairs administration of the Republic of Kazakhstan, Center of Gerontology of the Republic of Kazakhstan, Astana, Kazakhstan

Background: A Food Frequency Questionnaire (FFQ) is a limited checklist of foods and beverages with a frequency response section for subjects to report how often each item was consumed over a specified period of time. Calculations for nutrient intake can be estimated via computerized software programs (FETA) that multiply the reported frequency of each food by the amount of nutrient in a serving of that food.

Objectives: To assess the relationship between human microbiome and consumed food using FFQ;

Methods: Patients from 40 to 75 years old with and without metabolic syndrome answered food frequency questionnaire. Total number of participants was 84. Questions covered all food preferences, daily intake number of fruits, beverages, milk products, etc. More than 42 food products are listed in the survey. Finally, the validation of assessed FFQ FETA was conducted on a group of 84 individuals.

Results: Regarding results of FFQ, it was determined that people with metabolic syndrome consume daily food and beverages 1.5 times more than average daily dose. Comparing with healthy individuals each vitamin and mineral was checked for the average daily dose. In conclusion, Possible links of daily food products to the microbiome of human were found out. For example, The observed Vitamin D intake for the majority of the group was inadequate, i.e., in each applied assessment over 85% of the group was characterized by intake values lower than the recommended 5.0 μg of vitamin D day according to Daily recommendations.

PT192 Rhizobial biogeography in rooibos (aspalathus linearis): functional benefits in nutrient-poor soils

Josep Ramoneda[1], beat frey[2], johannes le roux[3], hannes andres gamper[4], emmanuel frossard[5]

[1]*ETH Zürich, D-USYS, Zürich, Switzerland*
[2]*Rhizosphere Processes Group*
[3]*Department of Biological Sciences*
[4]*Institute of Life Sciences*
[5]*Agricultural Sciences, D-USYS*

Background: Rhizobia establish mutualistic symbioses with legumes, providing fixed atmospheric N_2 in exchange for photosynthates. These functions are particularly important in habitats with low nutrient availability such as the arid Cape Floristic Region of South Africa. Rooibos is an endemic legume shrub from this region, growing in acidic and extremely poor soils in terms of available N, P, micronutrients, and water.

Objectives: This study aimed at determining the functional links between rhizobial diversity and plant nutrition using rooibos as a model case. We addressed two important questions: 1) Do cultivated and wild rooibos populations host distinct rhizobial assemblages? 2) If so, can we use this rhizobial diversity to improve the N nutrition and growth of rooibos?

Methods: In this study we increased the diversity of potential rhizobial partners of rooibos through mixing of soils from cultivated and wild populations. We raised rooibos seedlings from a commercial cultivar in cultivated, wild, and 1:1 mixes of both soils collected across its distribution range. We recorded plant biomass and nutrient accumulation, and described the rhizobial nodule communities through sequencing of functional (nodA) and taxonomic markers (gyrB).

Results: The strongest predictor of rhizobial community structure and diversity was the geographical origin of the soil, while the rhizobial communities from cultivated and wild rooibos were only marginally distinct. Yet, wild and mixed soils from a particular area increased plant growth compared to the other soils. Finally, we identified candidate rhizobial strains only found in that geographical area that might be driving such functional benefits.

PT193 Accurate assignment of oral taxa to health and disease will simplify early diagnosis of microbial dysbiosis leading to periodontitis

Magdalena Pavlikova[1], Lucie Najmanova[1], Jaroslav Mysak[2], Barbora Tesinska[1], Jiri Janata[1]

[1]Institute of Microbiology of the CAS, v. v. i., Laboratory for Biology of Secondary Metabolism, Prague, Czech Republic
[2]Department of Stomatology, First Faculty of Medicine, Charles University and General University Hospital in Prague, Prague, Czech Republic

Background: Periodontitis is a serious inflammatory disease affecting supporting structures of teeth (periodontium). Bacteria play an essential role in the disease development, they accumulate in the subgingival space and form biofilms. Depending on the velocity of progression of the tissue destruction we distinguish aggressive and chronic type of periodontitis. Probability of development of chronic periodontitis increases with the age of patient. The oral taxa present in patients with periodontitis are generally well described. The characterization of the oral microbiome associated with periodontal health, however, could be radically improve. In addition to that, the most important would be the definition of the shift from the healthy microbiome to disease.

Objectives: The aim of the project is characterization of oral microbiome in typically healthy persons on group of 100 young people (students of stomatology with excellent oral hygiene). We suppose, that in group of these very young people there is the biggest probability to obtain bacterial profiles without changes leading to periodontitis. Better definition of oral microbiome associated with periodontal health will subsequently enable the use of sequencing methods for early diagnosis of patients with periodontitis.

Methods: PCR, Illumina MiSeq sequencing, bioinformatics, HOMD 16S rDNA identification

Results: According to our preliminary results, oral microbiome in young healthy people differs from microbiome in middle aged periodontally healthy people. Based on our results, will be able to identify oral taxa typical for oral health.

PT194 Prevalence of Gut Fungi within the Segamat Community, Malaysia

Marie Andrea Laetitia Huët[1], Sui Mae Lee[1], Joash Ban Lee Tan[1]

[1]*Monash University Malaysia, School of Science, Subang Jaya, Malaysia*

Background: Most gut mycobiome studies focused primarily on diseased or hospitalised cohorts. To date, no such studies have been performed from the Malaysian healthy community. Fungal diseases are becoming a serious threat to human health and an exponential rise in number of antifungal resistant fungi within human body has been observed. A better understanding of the role and diversity of the mycobiome is critical.

Objectives: This research project seeks to characterize and investigate fungi isolated from the gut of Malaysians, within the community of Segamat, while assessing their biofilm production ability and antifungal resistance.

Methods: Four ethnic groups were targeted namely the Chinese, Indian, Malay and Jakun. Fecal samples and data collection were performed. Each stool sample was processed and screened for fungi and the isolates were subjected to morphological, biochemical and molecular characterisation, followed by biofilm production and fluconazole susceptibility testing. Risk factor analysis was also conducted.

Results: 233 fecal samples were collected. Prevalence of fungi among the different ethnic group targeted were as follows; 53.62% among Chinese participants (n=69), 49.9% within the Indian group (n=55), 46.30% and 27.27% among the Malay_(n=54) and Jakun_(n=55) cohort respectively. *Candida* species dominated the samples. Furthermore, Malay participants had significant higher yeast density ($P<0.05$) compared to the other ethnic groups and participants who do not regularly consume coffee had higher yeast load ($P<0.05$). We hypothesize that it might be due to differences in lifestyles. Moreover, isolates were weak biofilm producers and fluconazole resistant yeasts were detected among all the different ethnic group targeted.

PT195 Evaluation of brand-new iSeq 100 for metagenomics applications

Esther Malv[1], Aili Kallastu[1], Madis Jaagura[1], Jekaterina Kazantseva[1]

[1]*Center of Food and Fermentation Technologies, Tallinn, Estonia*

Background: A new iSeq 100 benchtop sequencing system, recently launched by Illumina, has been developed with a focus on both accuracy and cost-efficiency. The platform represents a great example of a tool that can be afforded and used routinely for multiple molecular profiling assays.

Objectives: Development of affordable and quick pipelines of next generation sequencing (NGS) for everyday usage in microbiological laboratory that enables effective resolving of assigned tasks and simplifies the whole scientific process.

Methods: We have successfully established iSeq 100 usage with the currently available i1 kit performing 2x150 bp reads for the whole genome sequencing (WGS) of small bacterial genomes as well as the 16S rRNA gene amplicon sequencing of different bacterial communities from human sources, various fermentation processes, bacterial cultures or environmental samples. We have developed in-house protocol starting from the gDNA extraction through the optimization of the library preparation to the bioinformatic analysis for various metagenomics analysis.

Results: Thorough optimisation of 16S sequencing protocols for specific applications allow above 92% Q30 quality score and high consistency of run-to-run reproducibility. Comparative analysis of a microbiome sample by WGS and various protocols for 16S sequencing shows reliability of received data. Standardisation and validation of library preparation, sequencing and bioinformatic analysis strategies allow using iSeq 100 platform for wide range of application, where quick and cost-effective response is crucial. However, all steps of the WGS and 16S sequencing pipeline need careful consideration because of the significant influence they have on the profiles obtained from the samples.

PT196 Association between intestinal microbiota and tail-biting in pigs

Nassima Rhabi[1], Alexandre Thibodeau[1], Philippe Fravalo[1], Nicolas Devillers[2], William P Theriault[1], Guy Beauchamp[1], Luigi Faucitano[2], Guillaume Gauthier-Larivière[1], Sylvain Quessy[1]

[1]Faculty of Veterinary Medicine, Pathology and Microbiology, St-Hyacinthe, Canada
[2]Agriculture and AgriFood Canada, Sherbrooke Research and Development Center, Lennoxville, Canada

Background: Tail-biting is a serious behavior problem seen in pigs and is linked to substantial economic losses, affects animal welfare and raises food safety issues. Diet changes have been shown to partly decrease tail-biting, suggesting a possible association with the intestinal microbiota.

Objective: This study aimed to evaluate the association between tail-biting and intestinal microbiota in pigs.

Methods: In a commercial farm, the intestinal microbiota in biter and bitten pigs was compared to a negative control group (non-biter and non-bitten). The groups (12 pigs per group) were selected at the beginning of the growing/finishing phase by a target behavior analysis centered on tail-biting (biter group) and a score of damages caused to the tail (bitten group). During and after a tail-biting episode, fecal samples were collected for each animal. The pig's fecal microbiota was analyzed from DNA extracted from freshly collected fecal matter using amplicon sequencing of the V4 region of the genes coding for the 16S rRNA by Illumina MiSeq.

Results: The fecal microbiota beta diversity analysis revealed a significant difference between biter and bitten pigs in comparison to the negative control group in terms of structure and composition of the microbiota, where *Lactobacillus*, have been significantly higher ($p=0.001$) in the negative control group compared to other groups. No significant difference was observed on the alpha diversity.

PT197 Microbial contamination of photographic and cinematographic materials in archival funds

Sabina Purkrtova[1], Yulia Shulha[1], Nikola Kovacova[1], Dana Savicka[1], Hana Sykorova[1], Jana Kadava[1], Barbora Benetkova[2], Michal Durovic[2], Katerina Demnerova[1]

[1]University of Chemistry and Technology, Prague, Dept. Biochemistry and Microbiology, Praha, Czech Republic
[2]University of Chemistry and Technology, Prague, Dept. Chemical Technology of Monument Conservation, Praha, Czech Republic

Background: Microbial contamination of photographic and cinematographic materials in archival funds represents an enormous danger for their long-time maintainance. The isolation and identification of present microorganisms enables to develop and to apply the most efficient regardful means of disinfection.

Objectives: To isolate and identify microbial contamination of different kinds of photographic and cinematographic materials in three archival funds in Czech Republic in 2018-2019.

Methods: The surface of materials was swabed by a previously optimised method using dry polyurethan damper. Used dry dampers were soaked and/or agitated in sterile saline solution, which was subsequently inoculated and cultivated on agar media (MEA, DG18, 25 °C, 7-10 days; PCA, 30 °C, 3-5 days). The air contamination was examined with an aeroscope. The isolates of bacteria were subisolated (CSB, 30 °C, 1-2 days) and identified by MALDI-TOF MS using Autoflex Speed MALDI-TOF and Biotyper 3.1 (Bruker Daltonics). The moulds were identified phenotypically.

Results: The majority of all bacterial isolates (273 isolates) were grampositive cocci, often incident in human skin and oral bacteriome (*Staphylococcus* spp., *Streptococcus* spp., *Micrococcus* spp., *Dermacoccus* spp., *Kocuria* spp.). Also genera forming endospores were abundantly represented (*Bacillus* spp., *Paenibacillus* spp.). For gramnegative bacteria only few genera were detected as *Neisseria* spp., *Acinetobacter* spp. and *Pseudomonas* spp. The sporadically isolated moulds were presented by typical genera *Alternaria*, *Aspergillus*, *Rhizopus*, *Penicillium* or less frequent species as *Paecilomyces marquandii* and *Myxotrichum deflexum*. The work was supported by the project DG18P02OVV062 (2018-2022, MK0/DG) of Ministry of Culture Czech Republic.

PT198 Effect of a phytobiotic blend on intestinal microbiota diversity in piglets

Myrna Olvera García[1], Hector Leyva-Jimenez[1], Jarintzi Rico-Ruiz[1], Gonzalo Villar[1], Luis Miguel Gomez-Osorio[1], Alberto Casarín-Valverde[1], Alejandro Sanchez-Flores[2]

[1]Grupo NUTEC, Research and Development, Santiago de Querétaro, Mexico
[2]Instituto de Biotecnología UNAM, Unidad Universitaria de Secuenciacion Masiva y Bioinformatica, Cuernavaca, Morelos, Mexico

Background: Phytobiotics have been proposed as an alternative to replace antibiotics growth-promoters (AGP), due to their effect on increasing feed efficiency, gut health as well as developing innate immunity.

Objectives: A feeding trial was conducted to examine the effect of a phytobiotic blend PGI (ProinteGI™) on intestinal microbiota in piglets.

Methods: Twenty-one-day-old pigs were randomly allocated to 2 groups of 4 pigs (n=8) during 49 days. Two diets were tested (21, 42 and 70d post-weaning). A basal pellet diet was used as control and the same diet + 500 ppm of PGI. A longitudinal study of the composition and dynamics of fecal microbiomes was performed between diets using V3-V4 16S rRNA amplicon sequencing for the Illumina platform. We characterized the composition and structure of the swine intestinal microbiota using bioinformatic tools to calculate Alpha and Beta diversity indexes. The taxonomic annotation was performed using the Metaxa2 software.

Results: Bacteroidetes and Firmicutes were the predominant phyla in both treatments. The microbiota of piglets supplemented with PGI showed high abundance of genera such as *Prevotella, Ruminococcus, Lachnospiraceae* and *Faecalibacterium.* These have been related with prebiotic, complex nutrients assimilation and butyrate production. Butyrate has been associated with an intestinal anti-inflammatory effect as well as energy fuel by the enterocyte. This study suggests that adding PGI to the feed increase the diversity and dynamics of bacterial population resulting in an improvement of intestinal health in pigs.

PT199 Characterization of microbial communities from gut microbiota of hypercholesterolemic and normochlesterolemic subjects

Nicolas Saavedra[1], Cristian Morales[1], Camilo Rebolledo[1], Marcelo Rojas[2], Vinicius Maracaja-Coutinho[3], Alejandro Cuevas[1], Kathleen Saavedra[1], Pamela Serón[1], Fernando Lanas[1], Luis Salazar[1]

[1]Universidad de La Frontera, Center of Molecular Biology and Pharmacogenetics, Scientific and Technological Bioresource Nucleus, , Chile
[2]Universidad del Desarrollo, Center of Genetics and Genomics, Faculty of Medicine, Chile
[3]Universidad de Chile, Department of Biochemistry and Molecular Biology, Faculty of Chemical and Pharmaceutical Sciences, Chile

Background: The gut microbiota is composed of multiple microbiological ecosystems, being the bacterial communities the best-described group. These communities have been associated with some diseases, demonstrating through animal models the relation between gut microbiota and disturbances of lipid metabolism suggesting a potential role in hypercholesterolemia.

Objective: To identify microbial communities differentially distributed in the gut microbiota from hypercholesterolemic and normocholesterolemic individuals.

Methods: The present case-control study included 27 hypercholesterolemic and 30 normocholesterolemic subjects defined by serum Lipoprotein density low cholesterol (LDL-C) levels >160 mg/dL and <100 mg/dL, respectively. Demographic and clinical information were recorded. Moreover, blood sample was obtained to measure serum glucose and lipids. Stool samples were obtained to isolate microbial DNA, which was used to amplify the V3-V4 regions from the bacterial 16S rRNA gene. The synthesized libraries were sequenced on the MiSeq platform and the obtained sequences analyzed by using the Quantitative Insights Into Microbial Ecology 2 (QIIME2) pipeline and the Linear Discriminant Analysis Effect Size (LEfSe) tool.

Results: Body mass index ($p=0.006$), triglycerides levels ($p=0.0007$), total cholesterol ($p<0.0001$) and LDL-C ($p<0.0001$) were significantly higher in hypercholesterolemic compared to normocholesterolemic individuals. Regarding the analysis of microbial communities by sequencing, no differences in microbial diversity between the groups were observed. However, the LDA score showed abundance to the order Chromatiales and genus Methanosphaera in hypercholesterolemic subjects, while normocholesterolemic subjects have abundance of the class Betaproteobacteria, order Fusobacteriales, genus Victivallis and Faecalibacterium ($p<0.05$).

PT200 Antimicrobial resistance and associated genetic determinants of Aliivibrio finisterrensis, a species that greatly predominates in the gut microbiome of farmed Atlantic salmon (Salmo salar L.) in Tasmania

Josephine Hamlett[1], John Bowman[1]

[1]*University of Tasmania, Sandy Bay Campus, Tasmanian Institute of Agriculture, Hobart, Tasmania, Australia*

Background: Farmed fish are an increasingly significant source of protein, especially with the current rapid expansion of the global "blue economy". The nature of fish GI tract environments makes them ideal as reservoirs for antimicrobial resistance (AMR) due to the high bacterial density present.

Objectives: Analysis of microbiome sequence data (16S rRNA V1-V3) indicated *Aliivibrio finisterrensis* is the dominant species in the gut microbiome of commercially farmed Atlantic salmon in Tasmania. The capacity of *Aliivibrio finisterrensis* to act as a reservoir of AMR in farmed salmon was investigated.

Methods: AMR was assessed using antibiotic containing disks and also via growth minimum inhibitory concentration analysis. CSLI guidelines was used to establish breakpoints. Genome sequence drafts were obtained for 10 representative strains to determine candidate AMR gene determinants.

Results: Bacteria randomly isolated from salmon faecal samples demonstrated aminoglycoside and penam resistance, but were susceptible to chloramphenicol, sulphonamides and tetracyclines. *A. finisterrensis* isolates were found to be highly resistant to multiple antibiotic drug classes, including all those important for aquaculture prophylaxis except for chloramphenicol, oxolinic acid, furazolidone, rifampicin, and trimethoprim. The resistance profile of *A. finisterrensis* can be explained by the occurrence of multiple (n=38) genetic resistant determinants that were found to be distributed homogenously across genome-sequence defined strain clusters as well as other *Aliivibrio* species. The data strongly suggests much of the AMR intrinsic to *A. finisterrensis* and the genus was acquired through mostly old lateral gene transfer events. Normalised Resistance Interpretation, however, suggests more recent build-up of resistance, especially to trimethoprim.

PT201 Draft genome sequences of four multidrug resistant Acinetobacter baumannii isolates from two Romanian hospitals

Irina Gheorghe[1;2], Sarbu Ionela[2;3], Elvira Borcan[4], Murariu Carmen[5], Cristea Violeta Corina[6], Luminita Marutescu[2;7], Ilda Czobor[2], Carmen Curutiu[2;7], Yu Feng[8], Mariana Carmen Chifiriuc[2;7], Zong Zhiyong[8]

[1]*University of Bucharest, Faculty of Biology, Microbiology Department, Romania*
[2]*Research Institute of the University of Bucharest*
[3]*University of Bucharest, Faculty of Biology, Genetics Department*
[4]*Fundeni Institute*
[5]*Emergency Hospital for children "Marie S. Curie",*
[6]*Synevo Central Laboratory, Medicover Bucharest, Romania*
[7]*University of Bucharest, Faculty of Biology, Microbiology Department*
[8]*Center of Infectious Diseases, West China Hospital, Sichuan University, Chengdu, China*

Background: An increased incidence of carbapenem- and mulitdrug-resistant *Acinetobacter baumannii* nosocomial strains is reported lately, due to a high number of resistance and virulence genes located on mobile genetic elements (MGE).

Objectives: This is the first report on the draft genome analysis of four MDR *A. baumannii* strains isolated in Romanian hospitals from bloodstream infections, in two hospitals from Bucharest, Romania.

Methods: The genomes of four *A. baumannii* strains (1s, 14s, A7, A14) were sequenced using hiseqX Ten, Illumina (GenBank accession numbers: SRX4094320, SRX4094321, SRX4094322, SRX4094323). The paired reads were assembled by using SPADES 3.12.0 and the quality of assembling was checked by QUAST program. Addnotation was performed with RAST; the analysis of resistance and virulence profiles was achieved by using multiple programs (ResFinder, PlasmidFinderPathogenFinder, CARD, PubMLST, IS finder, Virulence Factor Database).

Results: All strains present resistance genes for beta-lactams (*bla*$_{\text{OXA-66}}$, *bla*$_{\text{OXA-72}}$, *bla*$_{\text{ADC-25}}$, *bla*$_{\text{TEM-1D}}$, and *bla*$_{\text{OXA-23}}$), aminoglycosides [aph(3')-VIa, ANT(3'')-IIa, aph(6)-Id, ANT(3'')-IIa, APH(3'')-Ib, aadA2, armA, aph(3')VIj, aph(3')-Ia, aadA1, aac(3)-Ia] and sulphonamides (sul1, sul2). Additionally, the 14s and A14 isolates carry resistance genes to macrolides, streptogramin B [msr(E), mph(E)], and tetracyclines (tetB, tetR). The genes involved in resistance to chloramphenicol, aminoglycosides, trimethoprim, sulphonamides, macrolides and tetracyclines were detected on MGE. All four strains harbor virulence markers associated with adherence, biofilm formation, enzyme and serum resistance, while some of them also contain genes involved in host immune system evasion (pgi, PMM, wza and weeH), iron uptake (entE, hemO) and regulation (abaIR).

Acknowledgments: we acknowledge the financial support of the Research Projects PN-III-P1.1-PD-2016-1798 (PD 148/2018).

PT202 Snapshot of resistance and clonality features in nosocomial and community acquired Acinetobacter baumannii strains

Irina Gheorghe[1;2], Sarbu Ionela[3], Cristea Violeta Corina[4], Elvira Borcan[5], Ilda Czobor[2], Carmen Curutiu[2;6], Yu Feng[7], Murariu Carmen[8], Veronica Lazar[2;6], Mariana Carmen Chifiriuc[2;9], Zong Zhiyong[10]

[1]University of Bucharest, Faculty of Biology, Microbiology Department, Research Institute of the University of Bucharest, Romania
[2]Research Institute of the University of Bucharest
[3]University of Bucharest, Faculty of Biology, Genetics Department, Research Institute of the University of Bucharest, Romania
[4]Synevo Central Laboratory, Medicover Bucharest, Romania, Romania
[5]Fundeni Institute, Romania
[6]University of Bucharest, Faculty of Biology, Microbiology Department, Romania
[7]Center of Infectious Diseases, West China Hospital, Sichuan University, Chengdu, China, China
[8]Emergency Hospital for children "Marie S. Curie",, Romania
[9]University of Bucharest, Faculty of Biology, Microbiology Department
[10]Center of Infectious Diseases, West China Hospital, Sichuan University, Chengdu, China

Background: Mortality rates associated with carbapenemase-producing *A. baumannii* (CPAB) isolates are steadily growing at present.

Objectives: We aimed to identify the dissemination of CPAB isolates in the community in Bucharest, capital city of Romania.

Methods: Thirty three CPAB clinical (n=30) and community acquired (3) isolates identified and tested by commercial systems and microdilution method (CLSI), recovered from the ICU patients and ambulatory (Sept. 2017-March 2018) have been analyzed. Genes encoding class D and B carbapenemases, as well as the presence of ISAba1 upstream of the carbapenemases were tested by PCR and sequencing. The clonality of CPAB was determined with PFGE and MLST (http://pubmlst.org/abaumannii/).

Results: 96.96% of *A.baumannii* isolates were resistant to imipenem, meropenem, ciprofloxacin. 63.63% of the isolates harboured $bla_{\text{OXA-51-like}}$, 66.66% revealed $bla_{\text{OXA-24}}$ and 33.33% carried $bla_{\text{OXA-23}}$. 45.45% had ISAba1, and 10 (30.30%) had ISAba1 immediately upstream of $bla_{\text{OXA-23}}$ gene. Nine major PFGE types were detected in the *A. baumannii* isolates, i.e.: pulsotype I (n=4), II (n=6), III (n=1), IV (n=3), V (n=1), VI (n=9), VII (n=5), VIII (n=1) and IX (n=1). MLST analysis showed that clone I, II, III and IX belonged to ST2, clone IV to ST312; clone V to ST642; clone VI and VIII to ST636; and clone VII to ST492. The presence of ISAba1 preceding the $bla_{\text{OXA-23}}$ gene might be an important mechanism of resistance transmission, suggesting that infections measures are required to reduce and control the dissemination of such resistant clones.

Acknowledgemts: we acknowledge the financial support of the Research Project PN-III-P1.1-PD 2016-1798 (PD148/2018).

PT203 Oleo gum resin of Boswellia serrata, A Potential Anti-inflammatory Agent: In Vivo study

Samadhan Dahikar[1], Sarita Bhutada[2]

[1]*Department of Microbiology, Sanjivani Arts, Commerce and Science College, Kopargaon, India*
[2]*Department of Microbiology, Sanjivani Arts, Commerce and Science College, Kopargaon, Kopargaon, India*

Background: Inflammation is a local response of living mammalian tissues to injury. It is a defense reaction exerted by the biological system in order to eliminate or limit the spread of injurious agent. *Boswellia serrata* oleo-gum resin, a traditional Ayurvedic remedy for inflammatory diseases.

Objectives: This study is aimed at investigating the anti-oxident, anti-inflammatory, and angiogenic activities of Oleo gum resin of Boswellia serrata**.**

Methods: In the present study resins were extracted and tested anti-inflammatory in in-vivo animal models and anti-oxidant activity. Wistar rats were divided into five groups, group 1 treated with carrageenan (control) whereas group 2, 3, and 4 treated with different doses (50, 100, and 200 mg/kg/bw) of B. serrata along with carrageenan, respectively. Group 5 treated with standard drug (Indomethacin 10 mg/kg/bw). Carrageenan induced paw edema and histopathological study of paw were evaluated in all experimental rats. Phytochemicals (secondary metabolites) present in the resin complex were analyzed qualitatively.

Results: Based on the results of the present study showed that Methanol, extracts of *B.Serrata* at the dose level 45 mg/kg and Hexane and Petroleum extract of *B.Serrata* at the dose level 180 mg/kg showed significant anti-inflammatory effect in wistar albino rats using carrageenan induced paw edema model. It was observed from the results that the resin of *B. serrata* showed more antioxidant activity. In vivo anti-inflammatory testing in the present study revealed Phytochemical constituent in the resins of *B. serrata* are blocking the inflammatory mediators, thereby reduces the inflammation.

PT204 Antioxidant response as a desiccation tolerance mechanism in iron-oxidizing acidophilic bacteria

Gloria Levicán[1], Claudia Muñoz-Villagrán[1], Sebastián Navarro[1], Gerardo Retamal-Morales[1]

[1]*Faculty of Chemistry and Biology, Department of Biology, Santiago, Chile*

Background: Acidophiles inhabit a broad variety of environments where are constantly exposed to drastic changes in water potential by desiccation, high osmolarity or freezing water. When living in acid drainages, these microorganisms are exposed to high concentrations of metals that impose high osmolarity and low water availability. To date, the study of desiccation tolerance has been addressed in neutrophilic bacteria, where an osmoprotective and antioxidant response have been reported, however, the response in acidophiles has not been described.

Objectives: The aim of this work was to characterize the involvement of antioxidant response against desiccation stress in *Acidithiobacillus. ferrooxidans* ATCC23270 and *Leptospirillum ferriphilum* DSM14647.

Methods: The effect of desiccation on cell viability and iron oxidation capability was measured by using Baclight Live/Dead system and determining oxidation-reduction potential of cell cultures. The content of reactive oxygen species (ROS) was determined by the fluorescent probe dichlorodihydrofluorescein diacetate. The mRNA levels were quantified by RT-qPCR.

Results: The resulted showed a significant reduction in cell viability and iron oxidation rate, and an increase in intracellular ROS content in cells were exposed to desiccated for 80 min. In addition, results indicated that pre-treating the cells with antioxidants (cobalamin for *L. ferriphilum* and glutathione for *At. ferrooxidans*) restored all stress parameters to levels exhibited by the control cultures. Finally, an increase in mRNA level of genes related to antioxidant response was also found in both microorganisms. Therefore, in acidophiles desiccation stress can lead to an increase in ROS generation and induce an antioxidant response as protective mechanism.

PT205 Anaerobic oxidation of ethane by marine archaea from hydrocarbon seeps

Chen Songcan[1], Niculina Musat[1], Lechtenfeld Oliver[1], Paschke Heidrun[1], Schmidt Matthias[1], Said Nedal[1], Popp Denny[1], Jaekel Ulrike[2], Florin Musat[1;2]

[1]*Helmholtz Centre for Environmental Research, Leipzig, Germany*
[2]*Max Planck Institute for Marine Microbiology, Bremen, Germany*

Background: Ethane is the second most abundant hydrocarbon in natural gas, amounting to over 10% by volume in gas of thermogenic origin. As a consequence, ethane is abundant at marine hydrocarbon sites impacted by natural gas seepage. Geochemical profiles of concentration and isotopic composition indicate microbial oxidation of ethane in anoxic sediment horizons. However, the microorganisms and biochemical mechanisms for anaerobic ethane oxidation are essentially unknown.

Objectives: identify anaerobic microorganisms able to oxidize ethane anaerobically, and elucidate the mechanism they use to channel ethane into their metabolism.

Methods: we applied anaerobic cultivation techniques to enrich ethane degraders from anoxic marine sediments. The obtained enrichment culture was investigated by amplicon sequencing, fluorescence in situ hybridization, and metagenome sequencing. Metabolites were analyzed by FT-ICRMS and LC-MS/MS, in correlation with synthesized standards.

Results: enrichment and consecutive transfers in fresh culture media over several years led to a sediment-free enrichment culture which oxidized ethane with sulfate as terminal electron acceptor. The enrichment was dominated by so-far uncultured Archaea related to the ANME-2d group. Other members of the culture were sulfate-reducing bacteria related to the marine SEEP-SRB1 group. In metabolite extracts we identified a mass peak corresponding to a hypothesized ethyl-coenzyme M; an identical mass peak and fragmentation pattern was obtained with an authentic standards. This finding was corroborated with the identification of a methyl-coenzyme M reductase-like gene in the archaeal metagenome. We conclude that the archaea activate ethane to ethyl-coenzyme M, as recently demonstrated for the oxidation of butane by *Ca.* Syntrophoarchaeum.

PT206 Resistance and virulence features in carbapenem-resistant community- acquired and nosocomial Acinetobacter baumanii isolates in Romania

Irina Gheorghe[1;2], Cristea Violeta Corina[3], Luminita Marutescu[1;2], Marcela Popa[2], Carmen Curutiu[1;2], Murariu Carmen[4], Trusca Bianca Simona[5], Elvira Borcan[5], Ghita Mihaiela Camelia[5], Veronica Lazar[1;2], Mariana Carmen Chifiriuc[1;2]

[1]*University of Bucharest, Faculty of Biology, Bucharest, Romania*
[2]*Research Institute of the University of Bucharest*
[3]*Synevo Central Laboratory, Medicover Bucharest, Romania*
[4]*Emergency Hospital for Children "Marie S. Curie", Bucharest, Romania*
[5]*Fundeni Institute*

Background: Carbapenem Resistant gram -negative bacteria represent an emerging cause of hospital acquired infections that pose a significant threat to public health. These bacteria are difficult to treat due to high levels of antibiotic resistance and are associated with high mortality.

Objectives: This study aimed to identify the virulence and antimicrobial resistance features in Carbapenem Resistant Acinetobacter (A.) baumannii (CRAB) strains isolated from hospital settings (H) and compare them with those isolated in the same period of time from community acquired (CA) infections in Bucharest, Romania.

Methods: The resistance mechanisms of 93 A*. baumannii* strains were characterized by phenotypic and genotypic methods.

Results: The antibiotic resistance profiles in *A. baumannii* isolates revealed high percentages of carbapenem-resistance in both H and CA isolates. The ciprofloxacin resistance was found very closed in both types of isolates (84%/83.33%). H and CA isolates revealed the intrinsec carbapenemase OXA-51and the acquired carbapenemases OXA-23, OXA-24, IMP,and VIM-2. The bla_{OXA-23} gene was identified in different plasmid types (GR2-Aci1, GR6-pACICU2). rep135040, p3S18 and Aci6 in H *A. baumannii* isolates. TOXA-51-like alleles corresponding to the two main sequence groups were identified as bla_{OXA66} (63.63% of the isolates) and respectively, bla_{OXA-69} (38.39%) and revealed the corresponding type ofompAand csuE sequence grouping. AphA6 (24%/16.6%), AphA1 (16%/16.6%) and aadB (9.3%/5.5%) genes were responsible for aminoglycosides resistance. Our survey revealed a high drug resistance in *A. baumannii* isolates. Different plasmid groups containing CRAB isolates may facilitate the bla_{OXA23} dissemination. Acknowledgments: we acknowledge the financial support of the Research Projects PN-III-P1.1-PD-2016-1798 (PD 148/2018).

PT207 Estradiol effect on vaginal Lactobacillus crispatus

Maximilien Clabaut[1;2;3], Coralie Kremser[4], Madina Karsybayeva[5], Jean-Pierre Picot[6], Aïna Queiroz[6], Chantal Pichon[7], Gérard Gredziniak[8], Pierre-Jean Racine[1;2;3], Sylvie Chevalier[1;2;3], Marc Feuilloley[1;2;3]

[1]*Laboratoire de Microbiologie, Signaux et Microenvironnement EA4312*
[2]*Université de Rouen , France*
[3]*Normandie Université, France*
[4]*GimoPharm, Longjumeau, France*
[5]*Remedials Laboratoire, Paris,*
[6]*IDbio Novacap group, Limoges, France*
[7]*Centre de Biophysique Moléculaire, CNRS UPR4301, Orléans, France*
[8]*Cosmetic Inventions, Antony, France*

Background: Lactobacilli is the major phylum of the normal human vaginal microbial community. Several studies have revealed that vaginal disorders and dysbiosis are associated to an important decrease of the Lactobacillii population and the emergence of pathogens. Many strategies have been proposed to protect the vaginal epithelium and re-equilibrate the microbiota to inhibit proliferation of undesired bacteria. Their real impact remains insufficient presumably because of the complex interactions between the microbiota and the host. Indeed, events such as menopause or child-birth are associated to both hormonal and microbiota variations. Moreover, a direct effect of estrogens on the vaginal microbiota has never been investigated.

Objectives: We choose to focus the present study on the potential effects of estradiol on *Lactobacillus crispatus*, one of the dominant species in the healthy vagina.

Methods: *L. crispatus* strains CIP104459 (Institut Pasteur) and V4 from vaginal swab origin were chosen to investigate the effects of estradiol on growth, adhesion, biofilm formation, auto-aggregation and biosurfactant production.

Results: This study revealed that CIP and V4 strains have different and variable behaviours in terms of biofilm forming potential, auto-aggregation and biosurfactant production. Noticeably, estradiol was shown to modulate these activities. In addition, *in silico* studies suggest that the effects of estradiol on *L. crispatus* could be explained by the expression of ortholog(s) of the human estradiol receptor allowing bacteria to adapt their physiology in response to estradiol.

PT208 Type VI secretion system and expression of flagellar class IV genes in the Pseudomonas fluorescens MFE01 strain

Mathilde Bouteiller[1], Gallique Mathias[1], Kosta Artemis[2], Hardouin Julie[3], Konto-Ghiorghi Yoan[1], Barbey Corinne[1], Latour Xavier[1], Chane Andréa[1], Feuilloley Marc[1], Merieau Annabelle[1]

[1]*Laboratoire de Microbiologie Signaux et Microenvironnement, Evreux, France*
[2]*Plateforme de Microscopie de l'Institut de Microbiologie de la Méditerranée, Marseille, France*
[3]*PISSARO proteomics facility, Mont-Saint-Aignan, France*

Background: Type VI secretion systems (T6SSs) are multiprotein contractile nanomachines that inject toxic effectors into prey cells. Nowadays, the most studied effectors exhibit antibacterial activity acting on peptidoglycan, lipid membrane or DNA.

We previously reported that the *Pseudomonas fluorescens* MFE01 strain possess an antibacterial activity and can immobilize competitive bacteria, thanks to its T6SS. Hcp1 (hemolysin co-regulated protein 1) is involved in this inhibition of motility. We observed that mutation in *hcp1* gene results in loss of flagella production in MFE01Δ*hcp1* strain.

In *Pseudomonas* genius, flagella are extracellular appendages that are assembled in a four-tiered transcriptional regulatory circuit. The flagellar class IV genes are expressed by the FliA sigma factor that is release after the secretion of FlgM (anti-sigma factor) through incomplete flagellar apparatus.

Objectives: The aim of this study is to identify a new Type 6 secretion system effector that acts on bacterial motility.

Methods: Here, we combine transcriptomic and proteomic analysis, on the wild type and MFE01Δ*hcp1* deletion mutant to understand the loss of flagella. We introduce *fliA* gene in MFE01Δ*hcp1* to confirm our hypothesis. In order to identify this new effector, we used co-immuno-precipitation.

Results: In MFE01Δ*hcp1* mutant, we observed a down-expression of flagellar class IV genes, which may be explain by the lack of the anti-sigma factor (FlgM) secretion. The overexpression of the sigma factor FliA restores the motile phenotype.

PT209 Exploring acyclic boronic derivatives as potent beta-lactamases inhibitors with broad spectrum activity

Lorenzo Maso[1], Antonio Quotadamo[2], Pierangelo Bellio [3], Martina Montanari[2], Giuseppe Celenza [3], Maria Paola Costi [2], Donatella Tondi[2], Laura Cendron[1]

[1]*University of Padua, Department of Biology, Italy*
[2]*University of Modena and Reggio Emilia, Department of Life Sciences, Italy*
[3]*University of L'Aquila, Department of Biotechnological and Applied Clinical Sciences, Italy*

Background: Bacterial resistance has become a worldwide concern after the emergence of pan-resistant clinical isolates. beta-lactamases enzymes (BLs) represent one of the major mechanisms of bacterial resistance. Among them, the metallo dependent subclass (MBLs) is particularly worrisome, given their capability of hydrolysing beta-lactam antibiotics as well as inhibitors currently in use. In particular, New Delhi metallo-BL-1 (NDM-1), the most prevalent type, is extremely efficient in inactivating nearly all-available antibiotics including last resort carbapenems. While for serine-beta-lactamases (SBLs) inhibitors are available in therapy, for MBLs no inhibitor have been at the present approved, compromising the efficacy of treatments of bacterial infections.

Objectives: Our study aimed to characterize promising β-lactamases inhibitors with broad spectrum activity toward both serine and metallo BLs. We focused our attention on boron based benzothiophene derivatives and used a small library, based on such scaffold, to develop non-beta-lactam-like compounds able to bind BLs active site and inhibit their activity. Our choice has been encouraged by the recently approved serine BLs inhibitor vaborbactam and promising studies on cyclic boronic inhibitors.

Methods: We performed an in depth structural and mechanicistic study of a small library of acyclic boronic benzothiophene derivatives with broad spectrum (both SBLs and MBLs) activity.

Results: In solution kinetic characterization as well as extensive X-Ray crystallographic analysis of our best candidates have been performed and will be presented here.

PT211 Characterization of a novel three-component toxin-antitoxin module from the pathogenic bacterium Escherichia coli O157:H7

Pieter De Bruyn[1;2], Alexandra Vandervelde[1;2], Dukas Jurenas[3], Laurence Van Melderen[3], Remy Loris[1;2]

[1]Vrije Universiteit Brussel, Center for structural biology, Ixelles, Belgium
[2]Vlaams Instituut voor Biotechnologie, Elsene, Belgium
[3]Cellular and Molecular Microbiology, Faculté des Sciences, Université Libre de Bruxelles, Gosselies, Belgium

Background: The *paaR2-paaA2-parE2* operon is a unique three-component toxin-antitoxin module present in pathogenic *Escherichia coli* O157:H7 and a possible target for the development of new antibacterial drugs (Hallez et al., 2010; Sterckx et al., 2016). Aside from the toxin (ParE2) and antitoxin (PaaA2), the operon encodes an additional regulator (PaaR2) that is involved in the regulation of the transcription of the operon.

Objectives: The objective of this work is to understand how PaaR2 autoregulates the transcription of the operon and if this regulation is possibly linked to the onset of persistence.

Methods: Via a combination of structural biology, biochemistry and biophysical experiments, a mechanistic model for the autoregulation of the *paaR2-paaA2-parE2* operon is constructed.

Results: PaaR2 and an adjacent repressor have been found to regulate transcription of the *paaR2-paaA2-parE2* operon and of a neighboring operon in a mechanism that resembles the CI-Cro repression mechanism from bacteriophage λ.

References:

Hallez, R., Geeraerts, D., Sterckx, Y., Mine, N., Loris, R., & Van Melderen, L. (2010). New toxins homologous to ParE belonging to three-component toxin-antitoxin systems in Escherichia coli O157:H7. Molecular Microbiology, 76(3), 719–32.

Sterckx, Y. G.-J., Jové, T., Shkumatov, A. V., Garcia-Pino, A., Geerts, L., De Kerpel, M., Lah, J., De Greve, H., Van Melderen, L., Loris, R. (2016). A unique hetero-hexadecameric architecture displayed by the E. coli O157 PaaA2-ParE2 antitoxin-toxin complex. Journal of Molecular Biology, 428(8):1589-603.

PT212 Activity, neutralization and regulation of acetyltransferase toxin AtaT

Dukas Jurenas[1;2], Laurence Van Melderen[2], Abel Garcia-Pino[2]

[1]*Centre national de la recherche scientifique (CNRS), Laboratoire d'Ingénierie des Systèmes Macromoléculaires (LISM), Marseille, France*
[2]*Université Libre de Bruxelles, Département de Biologie Moléculaire, Gosselies,*

Background: Toxin antitoxin (TA) systems are small genetic elements encoded in bacterial mobile genetic elements (MGE) and chromosomes. TAs are involved in stable inheritance mechanisms by inhibiting bacterial growth in the daughter cells that are losing the TA-bearing MGEs. Chromosomal TAs are thought to be involved in genetic programmes allowing to halt bacterial growth in unfavourable conditions. AtaT is a novel acetyltransferase toxin from AtaR-AtaT system from enterohemorrhagic Escherichia coli O157:H7.

Objectives: Study molecular mechanism of toxicity, neutralization and regulation of production of AtaT toxin.

Methods: AtaT toxicity mechanism was unraveled by in vivo and in vitro assays by tracking the transfer of acetyl moiety from Acetyl-CoA. Mechanism of neutralization and transcription regulation was detailed by obtaining crystal structures of antitoxin, toxin, and a TA complex bound to DNA. The specific interactions were confirmed in vivo and in vitro by mutantions designed based on structural information.

Results: AtaT blocks initiation of translation by acetylating initiator Met-tRNA^{fMet} on methionine and thereby prevents its interaction with initiation factor IF2 and presentation to 30S ribosomal initiation complex. AtaT is neutralized by AtaR directly after its translation and locked in an inactive monomeric state. Heterohexameric AtaR2-AtaT-AtaR2-AtaT complex is an effective transcription repressor for ataR-ataT operon. Excess of AtaT breaks down the complex to heterotetrameric form. The later complexes can no longer repress transcription but still neutralize the toxin. AtaT when released form initial TA complex dimerizes and two monomers come together to form large positively charged binding surface that specifically recognizes its target tRNA.

PT213 Bacillus subtilis RecA interacts with and loads RadA/Sms to promote branch migration during natural chromosomal transformation and DNA repair

Rubén Torres Sánchez[1], Ester Serrano Álvarez[1], Juan Carlos Alonso Navarro[1]

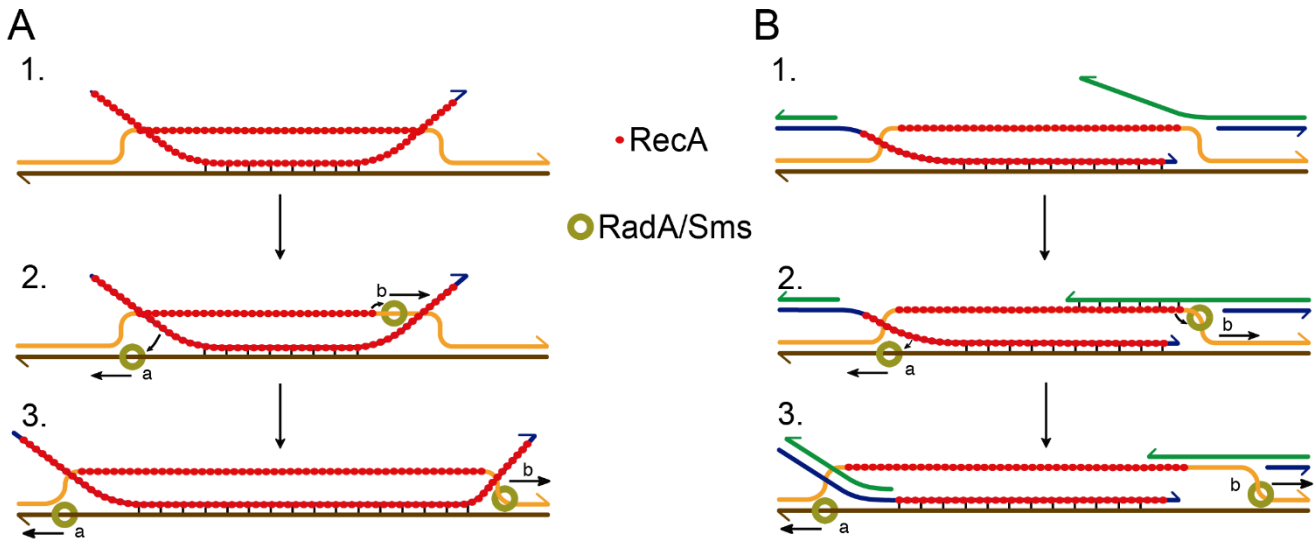
[1]*National Center for Biotechnology, Department of Microbial Biotechnology, Madrid, Spain*

Background: RadA protein, that has a RecA-like ATPase domain, is conserved from bacteria to plants, but its role is poorly understood. *Streptococcus pneumoniae* RadA is a 5'→3' DNA helicase involved in natural chromosomal transformation. Deletion of *Escherichia coli radA* renders cells marginally sensitive to DNA damaging agents. *E.coli* RadA acts as a branch migration translocase during RecA-catalysed strand exchange reaction, but no helicase activity has been documented.

Objectives: The main objective is to characterize *B.subtilis* RadA/Sms, together with the recombinase RecA, in natural transformation and DNA repair, processes that play a central role in bacterial evolution, survival and antibiotic resistance genes spreading.

Methods: *in vivo* and *in vitro* approaches were used: determination of transformation efficiency and sensitivity to DNA damaging agents of Δ*radA* and different RadA/Sms mutant variants in its Zn-binding (C13A, C13R) and Walker A (K104R) domains; and characterization of its interaction with other proteins, ATPase, DNA binding and helicase activities of RadA/Sms and its variants, in concert with RecA.

Results: Single-stranded (ss)DNA stimulates the ATPase activity of C13A and C13R, but not of *wt* RadA/Sms. *wt* and K104R, but not C13A and C13R RadA/Sms, inhibit ssDNA-bound RecA-mediated ATP hydrolysis. *wt* and C13A RadA/Sms unwind a 5´-tailed DNA in the 5'→3' direction. In the presence of RecA, *wt* RadA/Sms also unwinds a 3´-tailed DNA, but not C13A RadA/Sms, that cannot interact with RecA. We propose that RecA interacts with and recruits RadA/Sms onto ssDNA to facilitate D-loop extension in the 5'→3' direction, promoting chromosomal transformation (A) and DNA repair(B).



PT214 Two to tango? A curious case of twin type II plasmid-borne restriction-modification systems

Robert Lasek[1], Adrian Sówka[1], Dariusz Bartosik[1]

[1]*University of Warsaw, Faculty of Biology, Department of Bacterial Genetics, Warszawa, Poland*

Background: As a specific defense mechanism of prokaryotic cells against exogenous DNA, restriction-modification (R-M) systems present a decisive impact on horizontal gene transfer. Given their biological role they accommodate a number of functions: (a) the maintenance of the restriction activity against the exogenous DNA, (b) the inhibition of the potential "autotoxic" activity of endonucleases (REases), and (c) the maintenance of an adequate level of the host's DNA methylation by methyltransferases (MTases). Therefore they tend to be controlled by fine-tuned regulatory mechanisms.

Objectives: We investigated a curious case of the two closely related type II R-M systems identified in plasmid pP62BP1 of an Arctic strain of *Psychrobacter* sp. in order to characterize their activity and regulatory mechanisms.

Methods: We examined enzymatic activity of MTases and their DNA-binding properties in both in vitro and in vivo experiments. The transcriptional activity of the putative promotor sequences located within the systems was probed both in *E. coli* and *Psychrobacter* sp.

Results: The pP62BP1 R-M systems are controlled by a complex regulatory network, which appears to encompass not only the function of the MTases encoded therein (MTase-DNA interactions in regulatory sequences as well as cytosine methylation in 5'-CCNGG-3' pentamers influencing the activity of the component gene promoters), but also transcriptional interference dependent on the internal promoters located within the REase-encoding genes. The studied case constitutes a unique experimental set-up for the investigation of the regulatory cross-talk between two related R-M systems and its influence on the biology of their host plasmid.

PT215 Detection of Alginate Lyase and Ulvan Lyase Activity in Tropically Isolated Biodegraders

Mothafar Sadek[1], Sveltana Kotelnikova[1], Nikisha Bartholomew[2], Romany Nightingale[1]

[1]St. George's University, Microbiology and Immunology, St. George's, Grenada
[1]St. George's University, Microbiology and Immunology, St. George's, Grenada

Enzymes producing oligosaccharides are of interest for biotechnology because of their immune stimulating role in humans.

77 strains of gram-negative bacteria were isolated on brown- or green-algae-containing media (Bartholomew et al., 2018). Our hypothesis was that activity of ulvan lyase and/or alginate lyase will be detected in at least 80% of the tested newly isolated tropical strains.

The species we tested included *P. damselae, A. macleodii, E. hormaechi, P. gaetbulicola, P. putida, B. megaterium, V. panuliri,* and *P. leiognathi.* The strains were cultured in the marine mineral medium added with sodium alginate, 1 g/l or sodium ulvan, 1 g/l as a sole source of carbon and energy, separately and at duplicates at 30°C. DNS protocol was applied to determine presence of reducing sugars at 540nm and unsaturated sugars at 235nm following incubation for 24 hours and for 72 hours in 12-fold dilutions in triplicates using 96 well microplates VarioScan LUX Multimode reader (ThermoFisher Scientific). Variance and T-test analyses were performed using the MS Excel.

75% of isolates presented alginate- or ulvan lyase activity and produced reducing and unsaturated sugars. 62% showed both of the activities. Experimental data agreed with 80% of the genomic predictions. Both enzymes were present in *P. damsela, P. gaetbulicola, B. megaterium, P. putida and A. macleodii.* Alginate lyase activity was detected in *Vibrio panuliri. Enterobacter hormachii* showed partial activity. The genes coding for these enzymes can be cloned and supplemented in the diet for production of reduced oligosaccharides and enhancement of microbiota in human intestines.

PT216 Assessing Cofactor usage in Pseudoclostridium thermosuccinogenes via heterologous expression of Central Metabolic Enzymes

Jeroen Girwar Koendjbiharie[1], Richard van Kranenburg[1;2]

[1]*Corbion, Gorinchem,*
[2]*Wageningen University & Research, Laboratory of Microbiology, Wageningen, Netherlands*

Background: *Pseudoclostridium thermosuccinogenes* and *Hungateiclostridium thermocellum* are being studied for their potential to contribute to a more sustainable bio-based economy. Both species were shown previously to rely on GTP or pyrophosphate instead of ATP as cofactors in specific reactions of central energy metabolism for reasons that are not well understood yet.

Objectives: Painting a more complete picture of the cofactor usage in the central metabolism of *P. thermosuccinogenes*, to help understand why ATP, GTP, and pyrophosphate are used in parallel as cofactors.

Methods: Since it is often impossible to predict cofactor specificity from the primary protein structure, thirteen enzymes from *P. thermosuccinogenes* were cloned and heterologous expressed in *Escherichia coli* to assess the cofactor usage *in vitro*. The assays were conducted with heat-treated *E. coli* cell-free extract devoid of background activity to allow the quick assessment of a relatively large number of (thermophilic) enzymes.

Results: Following the results of the glucokinase, galactokinase, xylulokinase, and ribokinase assays, it seems that phosphorylation of monosaccharides by and large is mainly GTP-dependent. Some possible implications of this relating to the adenylate/guanylate energy charge are discussed. Besides the highly expressed pyrophosphate-dependent 6-phosphofructokinase, another 6-phosphofructokinase was found to be ATP and GTP-dependent, while no 6-phosphofructokinase activity could be demonstrated for a third. Both type I glyceraldehyde 3-phosphate dehydrogenases were found to be NAD^+-dependent, and further, acetate kinase, isocitrate dehydrogenase, and three enzymes predicted to be responsible for the interconversion of phosphoenolpyruvate and pyruvate (i.e. pyruvate kinase; pyruvate, phosphate dikinase; phosphoenolpyruvate synthase), were also assessed.

PT217 Structure and function of wxl domain from enterococcus species

Mahreen Hassan[1], Stephane Mesnage [1], Mike Williamson Williamson[1]

[1]Department of Molecular Biology and Biotechnology at the University of Sheffield, Molecular biology and Biotechnology, SHEFFIELD, United Kingdom

Background: WxL is a domain present in many surface proteins from gram-positive bacteria including Enterococcus, Listeria or Lactobacillus. It has been shown to be sufficient for binding to peptidoglycan, the major component of the bacterial cell wall.. The structure of the WxL domain and the mechanism underpinning its interaction with peptidoglycan are unknown.

Objectives: We sought to express and purify a soluble and folded WxL domain to study its structure using Nuclear Magnetic Resonance (NMR).

Methods: Small Wxl (TX6119) from a surface protein produced by *Enterococcus faecium* was expressed in *Escherichia coli* and purified. Structure predictions were done using the Phyre 2 server.

Results: A Small WxL was expressed in a soluble form and purified. 1-D NMR and gel filtration experiments showed that the isolated domain was partially folded. Structure prediction and comparison with structural homologues using the Phyre 2 server suggested that the WxL domain is likely to display a IgG-like domain fold with a beta sandwich structure. Interestingly, structure predictions suggest that the recombinant protein expressed would require three additional strands to complete the sandwich structure. We hypothesize that the Small WxL may need extra strand(s) to stabilise the domain, probably coming from other proteins encoded by the gene locus. The formation of a structurally stable WxL domain is expected to allow the assembly of functionally important proteins required for virulence.

PT218 Characterization of the 2-methyl-4-amino-5-hydroxymethylpyrimidine phosphate synthase from Legionella pneumophilia

Michael Paxhia[1], Diana Downs[1]

[1]*University of Georgia, Microbiology, Athens, United States*

Background: The pyrimidine moiety of thiamine (2-methyl-4-amino-5-hydroxymethylpyrimidine phosphate, HMP-P) can be synthesized by ThiC-like or Thi5p-like synthases. Thi5 proteins are less broadly distributed and are poorly characterized. Significantly, Thi5p (from *Saccharomyces cerevisiae*) is the first enzyme reported to use pyridoxal-5'-phosphate as a substrate rather than a cofactor. Expression of *S. cerevisiae THI5* resulted in conditional complementation of a *thiC* mutant of *Salmonella enterica.* This heterologous system provides a unique tool to probe the characteristics of the Thi5 family of enzymes. *Legionella pneumophilia* encodes a *THI5* homolog and this bacterial enzyme was characterized in this study.

Objectives: This study seeks to characterize the *Legionella pneumophilia* HMP-P synthase (*Lp*Thi5p) and determine what is required for, and affects its function *in* vivo and *in vitro.*

Methods: Function of *Lp*Thi5p *in vivo* is determined by complementation of an *S. enterica thiC* mutant in liquid media using standard techniques. Protein production, purification, and assay implementation followed standard protocols.

Results: *LpTHI5* complemented a *Salmonella enterica thiC* mutant on many carbon sources demonstrating this protein has HMP synthase activity *in vivo.* Several protein variants compromised complementation and identified residues that are important for full activity. Unexpectedly, when isolated from *Escherichia coli*, *Lp*Thi5p purified with a metabolite that could be converted to HMP upon incubation. Efforts to determine the starting metabolite and mechanism of conversion to HMP have supported the hypothesis that radical chemistry is involved. This work confirmed the enzymatic activity of *Lp*Thi5p *in vivo* and begins to define how conserved structural features impact that activity.

PT219 Identification of a novel regulatory mechanism by response regulator AgrR in the heavy metal resistant bacterium Cupriavidus metallidurans

Md Muntasir Ali[1;2], Kristel Mijnendonckx[1], Natalie Leys[1], Pieter Monsieurs[1], Daniel Charlier[2], Rob Van Houdt[1]

[1]*SCK•CEN, Mol, Belgium*
[2]*Vrije Universiteit Brussel, Ixelles, Belgium*

Background: Two-component systems (TCS) are the predominant form of signal transduction in bacteria and are typically composed of a response regulator and a sensor kinase. The system is generally activated by a phosphotransfer cascade from the sensor kinase to an aspartate residue of the response regulator. In the heavy metal-resistant bacterium *Cupriavidus metallidurans,* TCSs play role in sensing and responding to heavy metals.

Objectives: In this research, we identified and characterized a novel response regulator, AgrR, involved in increased silver resistance.

Methods: RNA-Seq, in-silico motif prediction and gene deletion studies confirmed the role of the AgrRS system in the acquired silver resistance. The response regulator AgrR, its phosphomimicking (D51E) and phosphodeletion (D51A) variant were cloned and purified. Electrophoretic mobility shift assays (EMSAs), DNase I footprinting and premodification binding interference assays provided important information.

Results: Upregulated genes identified through RNA-Seq were studied by deletion, which confirmed the indispensable role of AgrRS in silver resistance. The phosphodeletion variant of AgrR (D51A) showed significant binding with its own promoter region and that of one of the target genes. DNase I footprinting and premodification binding interference assays at the own control region identified a near palindrome sequence and specific bases that strongly interact with AgrR (D51A). This phosphorylation-independent activation of AgrR allows proposing a model where the specific mutation of AgrS in NA4S, as well as loss of AgrS in other silver-resistant mutants, increases the pool of intracellular non-phosphorylated AgrR. Subsequently, the non-phosphorylated AgrR activates the expression of genes required for increased silver resistance.

PT220 LeuO and YjjM: interplay of the two proteins with multiple ORFs in Escherichia coli

Tatiana Bessonova[1;2], Uliana Shvyreva[1;3], Sofya Garushyants[4;5], Maria Tutukina[1;4], Olga N Ozoline[1]

[1]*Institute of Cell Biophysics RAS, Pushchino, Russian Federation*
[2]*Moscow State University, Moscow, Russian Federation*
[3]*Institute of Protein Research RAS, Pushchino, Russian Federation*
[4]*Institute for Information Transmission Problems RAS, Moscow, Russian Federation*
[5]*Skolkovo Institute of Science and Technology, Moscow, Russian Federation*

Background: LeuO is one of the global regulators, involved in pathogenicity and virulence control in *Salmonella*. For *Escherichia coli*, SELEX was performed revealing more than 100 genes as potential LeuO targets, but still we do not know what the binding site for LeuO is, and how it overlaps with other transcription factors involved in pathogenicity control.

Objectives: To test the possibility of synthesis of several protein products from the *leuO* gene and to check if there is any cross-section between the targets for LeuO and YjjM, that is also involved in control of the *E. coli* motility and virulence, and has multiple products synthesized from one gene.

Methods: Gene-doctoring, Western-blot, qRT-PCR, multiple alignment and tree reconstruction, SELEX, ChiP-seq.

Results: We found synthesis of several LeuO forms from the plasmid containing the gene and its regulatory region, as we observed earlier for YjjM. Using western-blot analysis of proteins synthesized from the *E. coli* MG1655 chromosome with the his-tagged *leuO* gene we detected three isoforms of LeuO, corresponding to additionaal translation starts from the conserved Met48 or Met63. Synthesis of all three LeuO isoforms was inhibited in the absence of *yjjM* suggesting their interplay. Six virulence-related genes were found to be common targets for LeuO and YjjM based on SELEX and ChIP-seq, and were further confirmed by qRT-PCR. As such, LeuO may be one of the first examples of alternative coding in *E. coli* that may be a part of a mechanism of pathogenicity control.
The work was supported by RFBR 18-34-01006

PT221 Light-dependent hydrogen evolution in green microalgae as accimilation mechanism to nutrient deficiency

Elena Petrova[1], Galina Kukarskikh[1], Alena Volgusheva[1], Tatyana Krendeleva[1], Adil Bayzhumanov[1], Taras Antal[1]

[1]*Moscow State University, Faculty of Biology, Department of Biophysics, Moskva, Russian Federation*

Background: Hydrogenases are a large group of specific enzymes in bacterial and some eukaryotic microorganisms which catalyze the simple reversible reaction: $2H^+ + 2e^- \rightleftarrows H_2$. Most of hydrogenases are thought to be involved in redox/energy balancing and fermentative metabolism under anaerobic conditions. In green algae, the hydrogenase reaction is linked to the photosynthetic electron transport; its physiological role is not fully clear.

Objectives: Hydrogen evolution can be sustained for several days upon acclimation of algal cells to nutrient depletion in light. It is noteworthy that in natural environment, algae are often exposed to limitation of important macronutrients. The goal of this study was to shed light on hydrogen evolution as acclimation mechanism in algae to the combined action of nutrient and oxygen deficit, when cell faces extremely reducing conditions.

Methods: In order to fulfill a goal we compared acclimation capacity between *Chlamydomonas reinhardtii* mutant HydEF lacking hydrogenase activity, and corresponding control strain. Such important physiological characteristics as photosynthetic activity, starch accumulation/degradation, ATP content, and culture viability were assessed upon sulfur depletion.

Results: We showed that the presence of the active hydrogenase in the algal cell improves stability of photosystem II, facilitates glycolysis, and promotes culture viability. Noteworthy, the hydrogenase activity had no effect on energy supply (ATP). The obtained results suggest that the hydrogenase reaction provides acclimation benefits under nutrient deficiency mainly due to regulation of intracellular redox and pH balance, whereas photophosphorylation is affected to a lesser extent.

PT222 Alpha-helical KfrC protein is a linker between relaxosome and active partitioning complexes in RA3 plasmid from IncU group

Monika Mitura[1], Ewa Lewicka[1], Grazyna Jagura-Burdzy[1]

[1]*Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Department of Microbial Biochemistry, Warsaw, Poland*

Background: The RA3 plasmid from the IncU incompatibility group is a broad-host-range (BHR), low-copy-number replicon containing clusters of functionally related genes associated with replication, stability and conjugation. Within the stabilization module, the active partition system is proceeded by two genes encoding α-helical proteins: KfrA and KfrC. Their homologues are also encoded on other widely disseminated BHR plasmids from IncP-1 and PromA groups. Auxiliary function of Kfrs in partition process has been suggested for IncP-1 homologues. KfrAs, DNA binding proteins, have the ability to form long rod-like structures and interact with KfrC. Intriguingly, in case of RA3 plasmid their stabilization role seems to be species-dependent. Identification of putative KfrC partners may help to reveal the role it plays in biology of RA3 plasmid.

Objectives: We aimed to identify the KfrC interactants encoded by RA3 plasmid.

Methods: High-throughput search for KfrC interacting partners was initiated by RA3 library construction in pUT18C vector of Bacterial Adenylate Cyclase-based Two Hybrid system. Screening of newly constructed library was conducted in BTH101 *cyaA* strain containing pKNT25-*kfrC*.

Results: Screening of RA3 plasmid library with KfrC as a bait led to identification of strong interactions between KfrC and a coupling protein VirD4, an essential component of conjugation system responsible for the recruitment of the relaxosome to the mating pair formation apparatus. This suggests that KfrC protein may play a role in stable maintenance of the RA3 plasmid in the host population via association of the active partition with conjugation processes.

Funding: National Science Centre in Poland (grant 2015/17/B/NZ2/01160).

PT223 Activation of the ArcA Response Regulator of Salmonella Typhimurium in Hypochlorite Stress Condition

Carolina Elizabeth Cabezas Molina[1], Alan C Briones[1], Diego Lorca[1], Agustín Cofré[1], María S Baquedano[1], Claudia Paz Saavedra[1;2]

[1]Laboratorio de Microbiología Molecular, Facultad Ciencias de la Vida, Universidad Andrés Bello, Santiago, Chile
[2]Millennium Institute on Immunology and Immunotherapy, Facultad Ciencias de la Vida y Facultad de Medicina, Universidad Andrés Bello, Santiago, Chile

Background: The two-component system (TCS) are conserved mechanisms between species ranging from archaea to bacteria. These systems are composed by a sensor kinase (SK) and a response regulator (RR) which has DNA binding domains that regulates the gene expression. The SK are activated by environmental signals, such as acidity, osmolarity, nutrients, reactive oxygen species (ROS), among others. This causes the SK autophosphorylation and the transphosphorylation to the RR. In our laboratory, we work with TCS ArcAB of *Salmonella* Typhimurium (*S.* Typhimurium) which is required for the survival and response to oxidative stress. However, the genetic regulation under oxidative stress generated by hydrogen peroxide are independent of each members of the system and the regulation under hypochlorite are unknown

Objectives: We suggest that the RR ArcA of S. Typhimurium is activated and responds to hypochlorite independently of the ArcB sensor, regulating genes required for ROS resistance, metabolism and /or virulence.

Methods: To determine which genes are regulated by ArcA in hypochlorite, a global transcriptomic analysis of the Δ*arcA* and Δ*arcB* strains was performed, determining that ArcA effectively regulates genes independent of ArcB. These findings were validated by qPCR and, in addition, we evaluated the activation of ArcA in this same condition, by phosphorylation assays, using Phos-tag™ gels in Δ*arcB* strains and deficient in the generation of acetyl phosphate (Δ*pta*)

Results: ArcA responds and activates independently of its sensor kinase in response to hypochlorite, suggesting that the activation of this RR is through another protein, unknown to date.

PT224 skfA regulation and new insights on the onset of the sporulation in Bacillus subtilis

Alessia Vercio[1], Moreno Galleni[1], Alain Brans[2]

[1]*University of Liège, Centre for Protein Engineering - Biological Macromolecules, Liège, Belgium*
[2]*University of Liège, Centre for Protein Engineering - Protein Factory, Liège, Belgium*

Background: The spore killing factor operon (*skf*) of *B. subtilis* is expressed during the reversible stages of sporulation under nutrient-limited growth conditions. The first protein encoded by this operon (SkfA) acts as an antimicrobial agent that lyses non-sporulating bacteria in order to provide nutrients to sporulating cells and eventually delay this process.

It has been shown that *skf* is directly regulated by the transcription factor PhoP under phosphate-limiting conditions and by the phosphorylated form of Spo0A (Spo0A~P). Finally Spo0A~P also activates *skf* expression by relieving the repressor activity of AbrB at the transition phase.

Objectives: Due to the role of *skf* operon during the earlier stages of the sporulation, we aimed to verify if its expression can also be induced by other nutrient limitations and regulated by other factors potentially implied in the cell fate decision to sporulate.

In perspective, we aim to determine the *skf*A activity in several conditions and mutants by time-lapse microscopy and FACS. The aim being to have more information about the sporulation triggering and achievement.

Methods: In order to measure the *skf* operon activity, we followed the expression of the GFP reporter gene placed under the control of *skf*A. The growth of the *B. subtilis* recombinant strain and the expression level of the reporter GFP were followed under different nutrient limiting conditions.

Results: Our preliminary results confirmed that *skf*A is activated under glucose and/or nitrogen limiting conditions. We also showed that its activation can be switched off by the addition of the specific limiting nutriments.

PT225 A Study on Expression Levels of Some Genes in Mar Regulon in the presence of Urine

Defne Gümüş[1], Fatma Kal ayci Yüksek[1], Özlem Sefer[2], Emre Yörük[2], Gülşen Uz[2], Mine Anğ Küçüker[1]

[1]*İstanbul Yeni Yüzyıl Üniversitesi, Medical Faculty, Medical Microbiology, Turkey*
[2]*İstanbul Yeni Yüzyıl Üniversitesi, Molecular Biology and Genetics, Turkey*

Background: Host conditions are known to modulate virulence, antibiotic susceptibility and growth of bacteria. Once an infection begins, all host conditions mean the environment of the bacteria. The mar locus controls the intrinsic susceptibility of bacteria by altering the expression of multiple genes located on the bacterial chromosome which leads resistance to different groups of antibiotics.

Objectives: In the present study we aimed to investigate the effects of human urine on expression of some genes in mar regulon (marA, marB, ompF, acrA, acrB and tolC) of an E. coli (SPC105).

Methods: Urine sample taken from a healthy male individual was used as culture medium. Standard American Petroleum Institute (SAPI) medium supplemented with 30% (v/v) adult bovine serum was used as control. Quantitative polymerase chain reaction (qPCR) was used to determine the gene expression levels. Statistical analyses were done by Tukey's post hoc-test.

Results: The expression levels of marA (P=0.009), marR (p=0.002), acrA (p=0.001), acrB (p=0.005) and ompF (p=0.0005) were shown to be increased significantly in urine. However expression of tolC was found to be not affected (p=0.3). The up regulation of marR did not affect the down regulation of marA. Therefore we considered that, the expression of marA and marR were affected independently from each other. Besides it seems that, up regulation of marA shown to be affected the expression levels of acrA and acrB; while ompF was up regulated independently from marA.

PT227 Substrate and function specificity of capsular heptose synthesis enzymes of Campylobacter jejuni

Carole Creuzenet[1], Heba Barnawi[1], Chelsea Kubinec[1], Laura Woodward[2], James Naismith[3]

[1]*Western University, London, Canada*
[2]*Saint Andrews, Saint Andrews, United Kingdom*
[3]*Oxford, Oxford, United Kingdom*

Background: *C. jejuni* causes severe diarrhea in humans who ingest contaminated water or meat. Its capsule is an essential virulence factor and we showed that modified heptoses within the capsule are essential for its function. We elucidated the enzymatic pathways for heptose synthesis in 2 strains that produce slightly different heptoses and identified conserved epimerases and reductases that could be targeted for inactivation. We hypothesize that inhibitors of these enzymes could reduce chicken colonization, thus decreasing meat contamination and downstream human campylobacteriosis. This requires understanding their mechanism of action and substrate specificity.

Objectives: To determine the mechanism of action and susbtrate specificity of new heptose C3, C5 epimerases and C4 reductases via detailed structure function studies.

Methods: We modeled the enzymes' structures (Swiss-Prot), identified residues potentially involved in substrate binding or catalysis and determined their role in catalysis and substrate and product specificity using site directed mutagenesis and capillary electrophoresis. We crystallized the enzymes to determine their structure with and without substrate and help interprete our mutagenesis data.

Results: Key catalytic residues were identified, some differing from predictions based on well-studied hexose modifying enzymes. Several residues that govern heptose vs hexose substrate specificity were identified, which is important to enable development of inhibitors specific for heptose modifying enzymes. Finally, residues that govern C3 vs C5 heptose epimerization specificity were identified, which explains how highly similar enzymes perform different epimerization reactions. This work will support the design of appropriate inhibitors.

PT228 RecA regulation by RecU and DprA during Bacillus subtilis natural plasmid transformation

Ester Serrano[1], Begoña Carrasco[1], Juan Carlos Alonso[1]

[1]*National Center for Biotechnology (CNB), Microbial Biotechnology, Madrid, Spain*

Background: During natural transformation, the RecA protein, with the help of mediators, polymerizes onto the incoming single-stranded (ss) DNA. If the internalized linear ssDNA shares homology with the recipient DNA, RecA integrates the incoming ssDNA into the recipient genome (chromosomal transformation). In the absence of homology with the recipient genome, heterologous oligomeric plasmid ssDNA is established independently of RecA (plasmid transformation). RecA polymerized on plasmid ssDNA is deleterious, and modulators promote RecA disassembly. RecU, which is present in bacteria of the phylum Firmicutes, contributes to plasmid transformation by an unknown mechanism.

Objectives: Characterize RecU as a modulator of RecA during nucleoprotein filament formation and which factor(s) may reverse(s) RecU activity during natural transformation in *Bacillus subtilis*.

Methods: Chromosomal and plasmid transformation efficiency experiments were performed in different *B. subtilis* null mutants; the kinetics of RecA-mediated hydrolysis of ATP or dATP were measured in the presence and in the absence of mediators and modulators proteins, alone or in combination; and (iii) formation of SsbA-, RecU-, RecA-ssDNA complexes, alone or in combination, was measured by atomic force microscopy.

Results: RecU positively contributes to plasmid transformation and negatively contributes to chromosomal transformation by regulating RecA activities. After unsuccessful homology searches, RecU (in a non-catalytic mode) limits RecA polymerization onto heterologous (plasmid) ssDNA favoring plasmid transformation. DprA which contributes to RecA filament growth on any internalized ssDNA, interacts with RecU and antagonizes its inhibitory effects on RecA activities during chromosomal transformation.

PT229 Bacterial phosphatases of the PAP2 family active on membrane lipids: structures, catalytic mechanism and specificities

Frederic Kerff[1], François Delbrassine[1], Meriem El Ghachi[1], Nicole Howe[2], Alexandre Lambion[1], Thierry Touzé[3], Martin Caffrey[2]

[1]*University of Liège, InBioS, Centre d'Ingénierie des Protéines*
[2]*Trinity College Dublin, Membrane Structural and Functional Biology Group, Schools of Medicine and Biochemistry and Immunology, Ireland*
[3]*Université Paris-Saclay, Institute for Integrative Biology of the Cell (I2BC), France*

Background: In bacteria, membrane PAP2 proteins play major roles in the metabolisms of the phosphatidylglycerol membrane phospholipid, undecaprenyl-phosphate (C55-P) lipid carrier and lipopolysaccharides. C55-P is the lipid carrier used in the peptidoglycan synthesis as well as for the processing other surface carbohydrate polymers. C55-P is synthesized de novo by the UppS synthase or results from an active recycling. In both cases, the last step of the process is the dephosphorylation of undecaprenyl pyrophosphate (C55-PP) by C55-PP phosphatases. The PgpB type PAP2 enzymes from *E. coli* (ecPgpB) and *B. subtilis* (bsPgpB) dephosphorylate both the C55-PP and the phosphatidylglycerol phosphate (PGP).

Objectives: The goal of this study is to better understand the catalytic mechanism and identify the structural features responsible for the substrate specificity of PAP2 enzymes of the PgpB type.

Methods: The structures are obtained by x-ray crystallography using crystals grown using the lipid cubic phase and the vapor diffusion methods. Molecular modeling was used to gain information on additional enzyme-substrate complexes.

Results: We determine the structures of an inactive mutant of bsPgpB in complex with PGP, as well as a complex between an inactive mutant of ecPgpB and C15-PP, a shorter form of the C55-PP lipid carrier. These data combined with molecular modeling bring new information on the catalytic mechanism and substrate recognition of these enzymes and question the substrate induced fit mechanism proposed for the hydrolysis of PGP by ecPgpB. These results are of interest for the search of new antibiotics.

PT230 The small non-coding RNA IsrG modulates bacterial proliferation of Salmonella Typhimurium

María José Barros[1], Lillian Gabriela Acuña Olivares[1], Paula Nuñez[1], Fernanda Montt[1], Diego Peñaloza[1], Iván Luis Calderón Lizana[1]

[1]*Universidad Andres Bello, Laboratorio de Genética y Patogénesis Bacteriana, Santiago, Chile*

Background: *Salmonella* Typhimurium (STm) can alter their proliferation to increase their chances to survive when is exposed to dramatic environmental changes. Adaptation to these changes involves the regulation of gene expression, where the small non-coding RNAs (sRNAs) may play critical roles by basepairing with specific mRNA targets. IsrG is an island-encoded sRNA from STm that is expressed in both extracellular and intracellular cultures. *In silico* prediction analysis identifying *fic* as a putative target, a mRNA that encodes a protein involved in inhibition of bacterial replication.

Objectives: Analyze the role of IsrG in the bacterial proliferation of *Salmonella* Typhimurium

Methods: We constructed a strain lacking IsrG (*isrG*$^{-}$) by homologous recombination and the bacterial growth and replication was analyzed by growth curves and a double reporter system based on fluorescence dilution. The expression of the putative target *fic* was analyzed by qRT-PCR in different backgrounds; wild type, *isrG*$^{-}$ and two strains overexpressing IsrG from pBAD, with (pIsrG^{mut}) or without (pIsrG) point mutations at the predicted interaction zone.

Results: The *isrG*$^{-}$ strain exhibited slower growth and replicated at a slower rate as compared to the wild type. The *fic* mRNA levels significantly increased in extracellular cultures of *isrG*$^{-}$ strain. Conversely, in the case of pIsrG strain the *fic* mRNA levels significantly decreased and this effect was abolished in the pIsrG^{mut} strain. Based on these results, we conclude that the sRNA IsrG participates in the modulation of STm proliferation and negatively regulates the expression of *fic*, a gene involved in bacterial growth inhibition.

PT231 Role of Escherichia coli endopeptidases and DD-carboxypetidases in infection and regulation of innate immune response

Sathi Mallick[1], Joyjyoti Das[2], Jyoti Verma[1], Samatha Mathew[1], Tapas K. Maiti[1], Anindya Ghosh[1]

[1]*Indian Institute of Technology Kharagpur, Department of Biotechnology, Kharagpur, India*
[2]*Indian Institute of Technology Kharagpur, Advanced Technology Development Centre, Kharagpur, India*

Background: Low-molecular-mass (LMM) PBPs, involved in peptidoglycan recycling can also produce peptidoglycan fragments capable of activating an innate immune response in host. The phagocytic cells of the host, including macrophages, play primary roles in the defence against the invading bacterial pathogens.

Objectives: To investigate how LMM PBPs in Enterobacteriaceae play a role to elicit/evade innate immune responses during infections.

Methods: To address the problem, we deleted certain endopeptidases and DD-carboxypeptidases from *E. coli* CS109 either individually or in combination, and studied the viability of these mutants in macrophages. The ability of the infected macrophages to exert oxidative killing, expression of surface activation markers TLR2 and MHC class II, and the release of TNFα, were assessed.

Results: Immune responses were elevated in macrophages infected with DD-carboxypeptidase mutants but reduced for endopeptidase mutants. However, the NFκB, iNOS, and TLR2 transcripts remained elevated in macrophages infected with both mutant types. Overall, we have shown, under normal conditions endopeptidases have a tendency to elicit the immune response but their effect is suppressed by the presence of DD-carboxypeptidases. Conversely, DD-carboxypeptidases, normally, have a tendency to reduce immune responses, as their deletions enhanced the same in macrophages.

Therefore, we conclude that the roles of endopeptidases and DD-carboxypeptidases are possibly counter-active wild-type cells where either class of enzymes suppresses each other's immunogenic properties rendering an overall maintenance of low immunogenicity that helps *E. coli* in evading the host immune responses.

PT232 A LINK BETWEEN cAMP AND UNCOUPLER SENSITIVITY IN CORYNEBACTERIUM GLUTAMICUM

Natalie Wolf[1], Julia Schulte[1], Meike Baumgart[1], Michael Bott[1]

[1]*Forschungszentrum Jülich GmbH, Institute of Bio- and Geosciences, IBG-1: Biotechnology, Germany*

Background: The transcriptional regulator GlxR, a Crp homolog, plays a central role in the regulation of metabolism in the actinobacterium *Corynebacterium glutamicum*, because it activates or represses about 14% of all genes [1]. GlxR binds its DNA targets only in complex with the second messenger cyclic adenosine monophosphate (cAMP). cAMP is synthesized by the membrane-bound adenylate cyclase CyaB [2] and degraded by the phosphodiesterase CpdA [3].

Objectives: In this study, we investigated the consequences of a low intracellular cAMP level using the strain *C. glutamicum* Δ*cyaB*.

Methods: The mutant showed a growth defect when acetate was present either as sole carbon source or together with e.g. glucose. The inhibitory effect of acetate on growth was concentration-dependent and came along with a reduced membrane potential compared to the wild type. As acetate acts as an uncoupler, we also tested the protonophore carbonyl cyanide m-chlorophenyl hydrazine (CCCP) and found the Δ*cyaB* strain to be more sensitive than the wild type. Studies regarding the GlxR binding profile revealed remarkable differences of genome-wide GlxR binding between wild type and the Δ*cyaB* strain grown with glucose and glucose-acetate.

Conclusion: Our findings suggest that the acetate effect on the Δ*cyaB* strain could be due to a reduced capability of proton motive force generation at reduced cAMP levels.

[1] Kohl & Tauch (2009) J. Biotechnol. 143: 239-246
[2] Cha et al. (2010) Appl. Microbiol. Biotechnol. 85: 1061-1068
[3] Schulte et al. (2017) Mol. Microbiol. 103: 534-552

PT233 Regulation of the ISPpu9 insertion sequence transposition by small RNAs in Pseudomonas putida

Guillermo Gómez García[1], Renata Moreno[1], Fernando Rojo[1]

[1]*Centro Nacional de Biotecnología, CSIC, Biotecnología Microbiana, Madrid, Spain*

Background: Insertion sequences (IS) are mobile genetic elements that only contain the functions required for transposition and can insert at multiple sites. *Pseudomonas putida* KT2440, a model soil bacterium, has seven copies of an IS called ISPpu9 inserted into sequences known as "repetitive extragenic palindromes" (REP). ISPpu9 belongs to the IS110 family.

In a directional RNA-seq assay we observed that the gene coding for the ISPpu9 transposase, *tnpA*, is flanked by two small RNAs (sRNAs) that we named IS9s (sense sRNA) and IS9as (antisense sRNA), respectively

Objectives: We explored the possibility that *tnpA* expression could be regulated by the IS9s and IS9as sRNAs.

Methods: We determined the transcription start sites of *tnpA* and of the IS9s and IS9as sRNAs, and we developed ISPpu9 variants that allow measuring transposition rates in the absence or presence of these sRNAs.

Results: We found that the *tnpA* mRNA has a long 5' untranslated region (5'-UTR). The IS9as sRNA significantly overlaps the *tnpA* 5'-UTR. In turn, the IS9s sRNA, located in the same strand as *tnpA*, was transcribed from a promoter located downstream of *tnpA*. The IS9as and IS9s sRNAs were abundant and their sequence was highly complementary. On the contrary, *tnpA* mRNA abundance was very low.

The expression of *tnpA* and that of the two sRNAs was analysed under different situations and genetic backgrounds, including a mutant in the *hfq* gene and a *P. putida* strain known as F1, which lacks ISPpu9. The results obtained and our current regulation model will be presented.

PT234 Molecular epidemiology of the seventh pandemic vibrio cholerae in china across fifty years

Duochun Wang[1], ping zhang[1], fengjuan li[1]

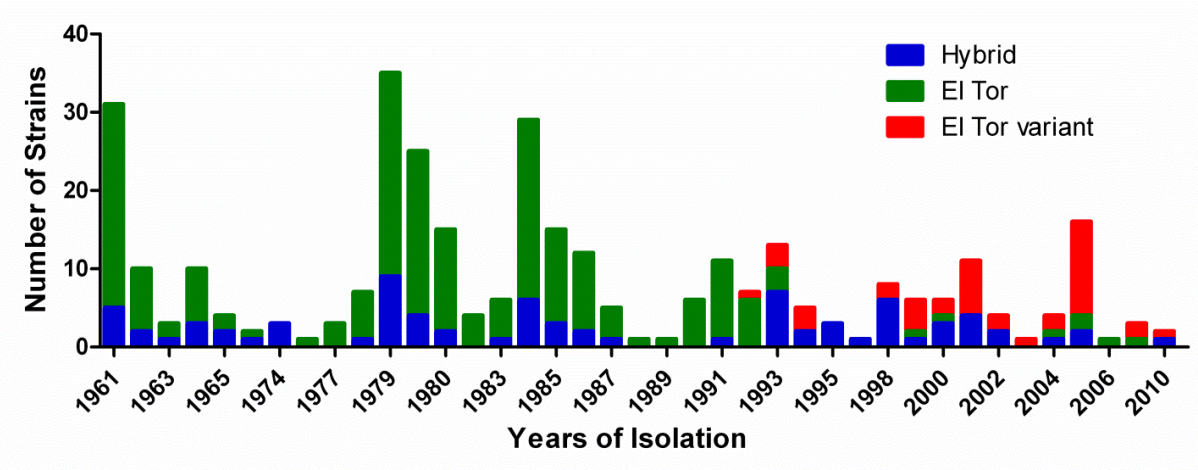
[1]*National Institute for Communicable Disease Control and Prevention, Chinese Center for Disease Control and Prevention, beijing, China*

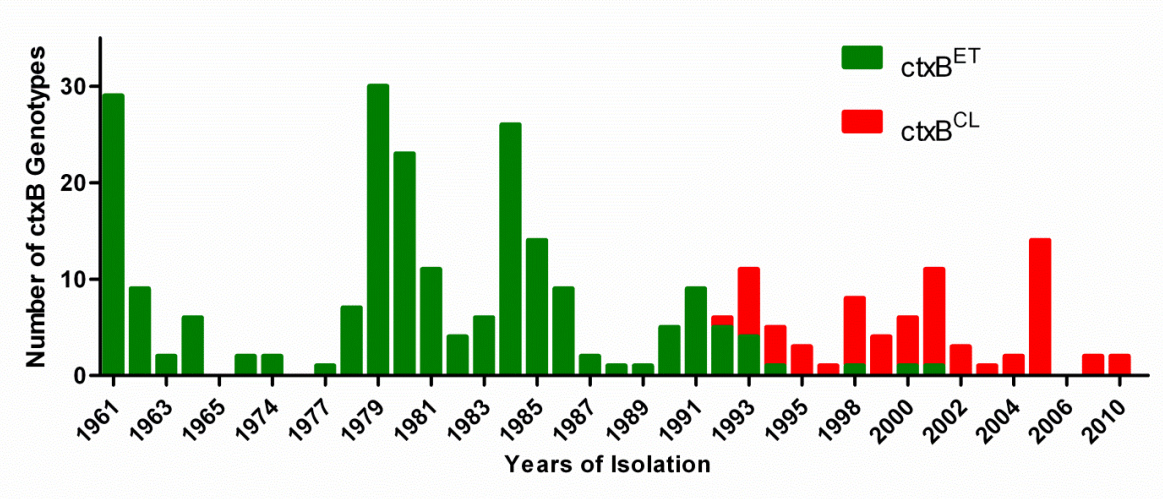
Background: *Vibrio cholerae* is the causative agent of the life-threatening diarrheal disease cholera. The sixth pandemic was caused by *V. cholerae* O1 of the classical biotype. The current seventh pandemic originated in 1961 and the causative agent is *V. cholerae* O1 of the El Tor biotype. In 1992, O139 cholera emerged in the coastal areas of India and then spread to many countries in Asia.

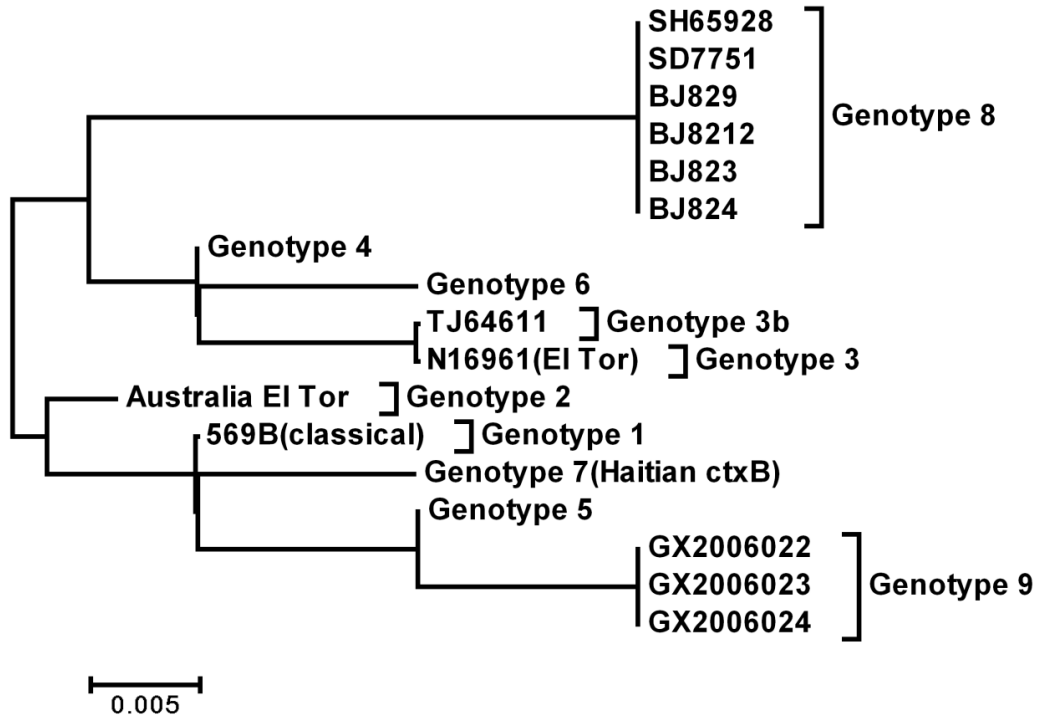
Objectives: To characterize phenotypic and genetic variant traits of *V. cholerae* over fifty years in China.

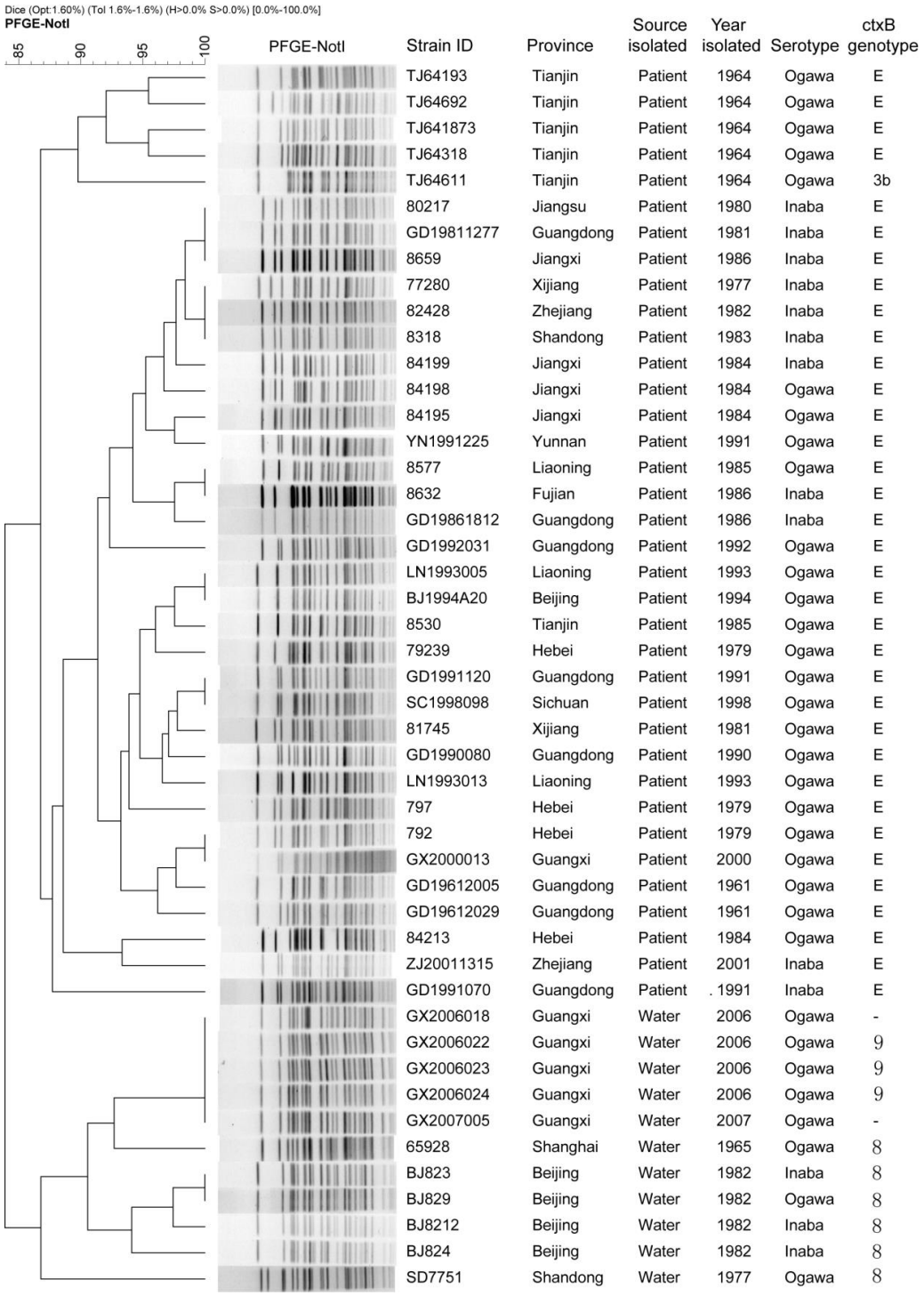
Methods: Phenotypic tests including polymyxin B susceptibility, chicken red cell agglutination and Voges-Proskauer reaction. PCR assays and sequencing analysis including the major virulence factors of *V. cholerae*. Molecular typing including pulsed field gel electrophoresis and multilocus sequence typing.

Results: During the seventh pandemic of *V. cholerae* O1 in China, the isolates of hybrid biotype (mixed classical phenotypes) were present during the entire 1961-2010 period, while El Tor genetic shifts (mixed classical genetypes) appeared in 1992 and replaced the prototype El Tor from 2002 to 2010. Meanwhile, several novel variants of *V. cholerae* O1 were identified, those novel variants possessed potential transmissibility and that they propagated in the local regions in China. *V. cholerae* O139, however, no change in classical forms has been observed. toxigenic *V. cholerae* O139 showing some diversity, have maintained a relatively tight clonal structure across a 20-year time span. Nontoxigenic isolates, in contrast, exhibited greater diversity, with multiple clonal lineages, than did their toxigenic counterparts.

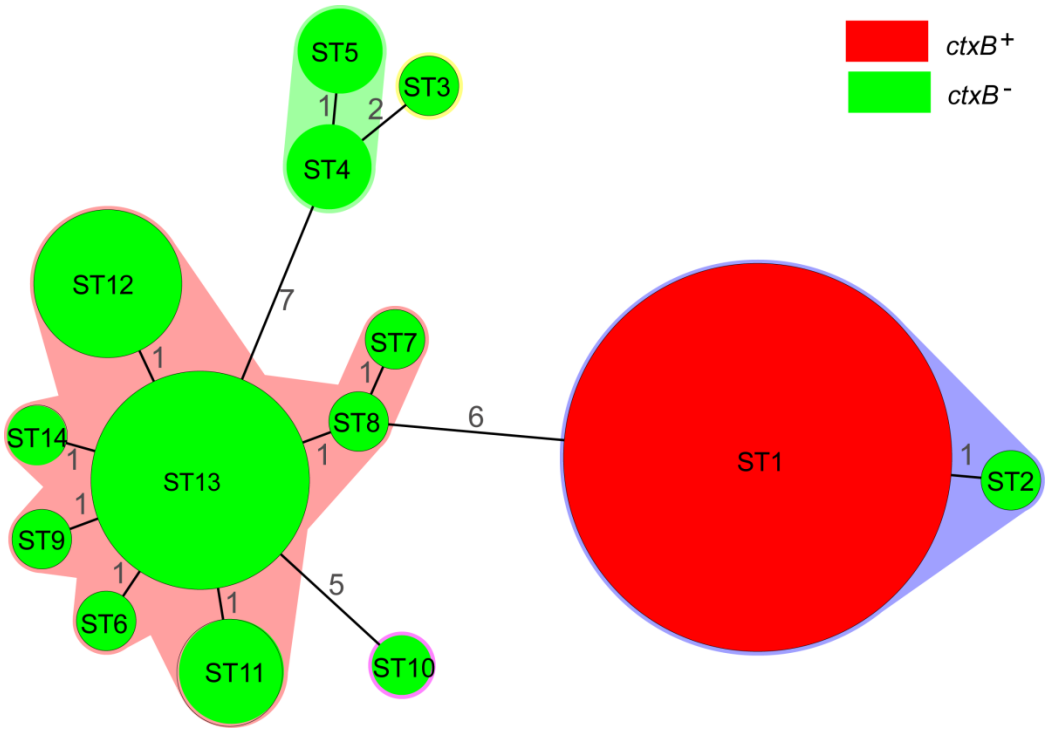


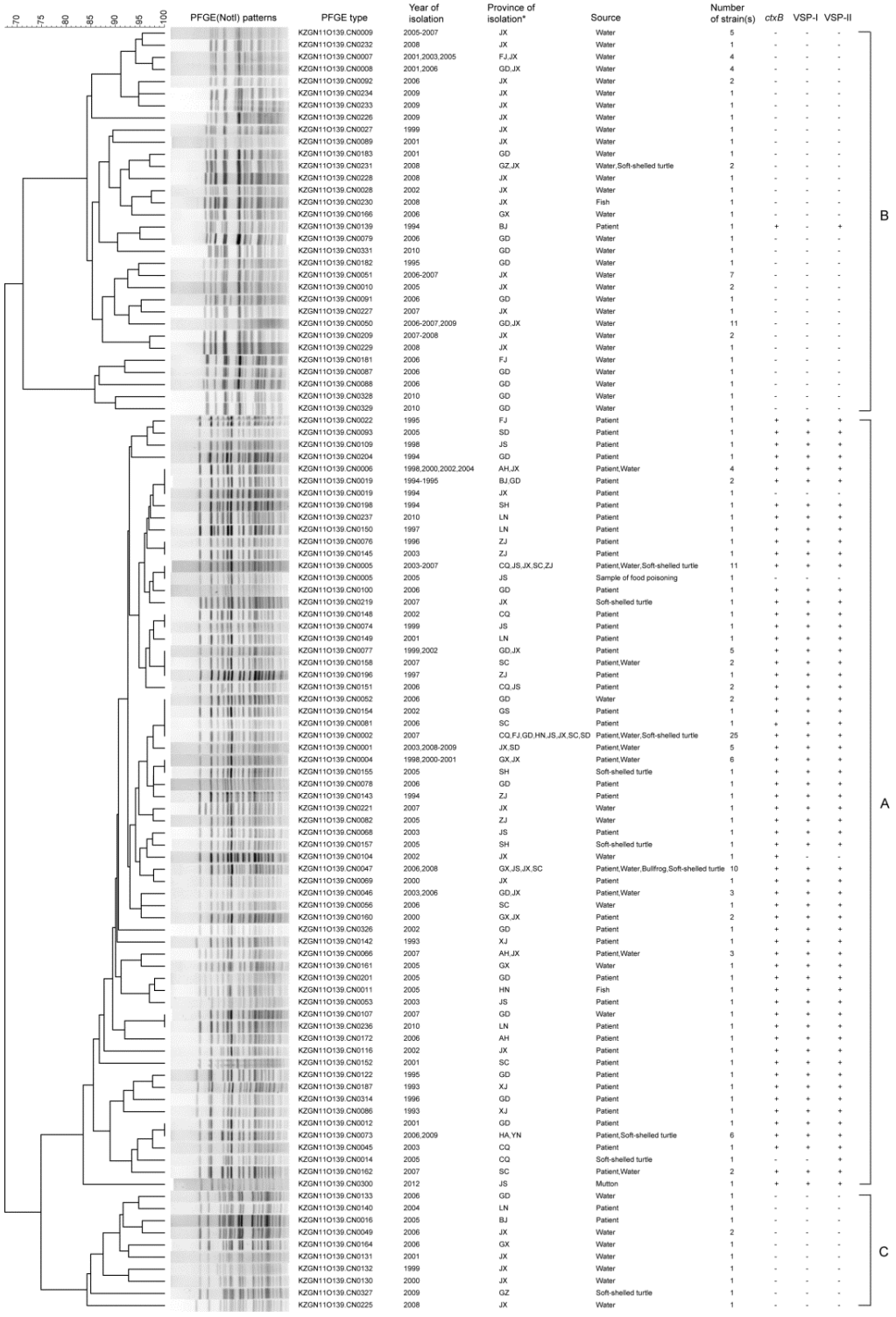

Number of ctxB Genotypes
30
20
10
0
ctxB^ET
ctxB^CL
1961
1963
1965
1974
1977
1979
1981
1983
1985
1987
1989
1991
1993
1995
1998
2000
2002
2004
2006
2010
Years of Isolation


SH65928
SD7751
BJ829
BJ8212
BJ823
BJ824
Genotype 8
Genotype 4
Genotype 6
TJ64611
Genotype 3b
N16961(El Tor)
Genotype 3
Australia El Tor
Genotype 2
569B(classical)
Genotype 1
Genotype 7(Haitian ctxB)
Genotype 5
GX2006022
GX2006023
GX2006024
Genotype 9
0.005



PFGE-NotI	Strain ID	Province	Source isolated	Year isolated	Serotype	ctxB genotype
	TJ64193	Tianjin	Patient	1964	Ogawa	E
	TJ64692	Tianjin	Patient	1964	Ogawa	E
	TJ641873	Tianjin	Patient	1964	Ogawa	E
	TJ64318	Tianjin	Patient	1964	Ogawa	E
	TJ64611	Tianjin	Patient	1964	Ogawa	3b
	80217	Jiangsu	Patient	1980	Inaba	E
	GD19811277	Guangdong	Patient	1981	Inaba	E
	8659	Jiangxi	Patient	1986	Inaba	E
	77280	Xijiang	Patient	1977	Inaba	E
	82428	Zhejiang	Patient	1982	Inaba	E
	8318	Shandong	Patient	1983	Inaba	E
	84199	Jiangxi	Patient	1984	Inaba	E
	84198	Jiangxi	Patient	1984	Ogawa	E
	84195	Jiangxi	Patient	1984	Ogawa	E
	YN1991225	Yunnan	Patient	1991	Ogawa	E
	8577	Liaoning	Patient	1985	Ogawa	E
	8632	Fujian	Patient	1986	Inaba	E
	GD19861812	Guangdong	Patient	1986	Inaba	E
	GD1992031	Guangdong	Patient	1992	Ogawa	E
	LN1993005	Liaoning	Patient	1993	Ogawa	E
	BJ1994A20	Beijing	Patient	1994	Ogawa	E
	8530	Tianjin	Patient	1985	Ogawa	E
	79239	Hebei	Patient	1979	Ogawa	E
	GD1991120	Guangdong	Patient	1991	Ogawa	E
	SC1998098	Sichuan	Patient	1998	Ogawa	E
	81745	Xijiang	Patient	1981	Ogawa	E
	GD1990080	Guangdong	Patient	1990	Ogawa	E
	LN1993013	Liaoning	Patient	1993	Ogawa	E
	797	Hebei	Patient	1979	Ogawa	E
	792	Hebei	Patient	1979	Ogawa	E
	GX2000013	Guangxi	Patient	2000	Ogawa	E
	GD19612005	Guangdong	Patient	1961	Ogawa	E
	GD19612029	Guangdong	Patient	1961	Ogawa	E
	84213	Hebei	Patient	1984	Ogawa	E
	ZJ20011315	Zhejiang	Patient	2001	Inaba	E
	GD1991070	Guangdong	Patient	1991	Inaba	E
	GX2006018	Guangxi	Water	2006	Ogawa	-
	GX2006022	Guangxi	Water	2006	Ogawa	9
	GX2006023	Guangxi	Water	2006	Ogawa	9
	GX2006024	Guangxi	Water	2006	Ogawa	9
	GX2007005	Guangxi	Water	2007	Ogawa	-
	65928	Shanghai	Water	1965	Ogawa	8
	BJ823	Beijing	Water	1982	Inaba	8
	BJ829	Beijing	Water	1982	Ogawa	8
	BJ8212	Beijing	Water	1982	Inaba	8
	BJ824	Beijing	Water	1982	Inaba	8
	SD7751	Shandong	Water	1977	Ogawa	8


ctxB+
ctxB-
ST5
ST3
ST4
ST12
ST7
ST8
ST14
ST13
ST9
ST6
ST11
ST10
ST1
ST2
1
2
7
1
1
6
1
1
1
1
1
5
1



PFGE(NotI) patterns	PFGE type	Year of isolation	Province of isolation*	Source	Number of strain(s)	*ctxB*	VSP-I	VSP-II	
	KZGN11O139.CN0009	2005-2007	JX	Water	5	-	-	-	
	KZGN11O139.CN0232	2008	JX	Water	1	-	-	-	
	KZGN11O139.CN0007	2001,2003,2005	FJ,JX	Water	4	-	-	-	
	KZGN11O139.CN0008	2001,2006	GD,JX	Water	4	-	-	-	
	KZGN11O139.CN0092	2006	JX	Water	2	-	-	-	
	KZGN11O139.CN0234	2009	JX	Water	1	-	-	-	
	KZGN11O139.CN0233	2009	JX	Water	1	-	-	-	
	KZGN11O139.CN0226	2009	JX	Water	1	-	-	-	
	KZGN11O139.CN0027	1999	JX	Water	1	-	-	-	
	KZGN11O139.CN0089	2001	JX	Water	1	-	-	-	
	KZGN11O139.CN0183	2001	GD	Water	1	-	-	-	
	KZGN11O139.CN0231	2008	GZ,JX	Water,Soft-shelled turtle	2	-	-	-	
	KZGN11O139.CN0228	2008	JX	Water	1	-	-	-	
	KZGN11O139.CN0028	2002	JX	Water	1	-	-	-	
	KZGN11O139.CN0230	2008	JX	Fish	1	-	-	-	
	KZGN11O139.CN0166	2006	GX	Water	1	-	-	-	B
	KZGN11O139.CN0139	1994	BJ	Patient	1	+	-	+	
	KZGN11O139.CN0079	2006	GD	Water	1	-	-	-	
	KZGN11O139.CN0331	2010	GD	Water	1	-	-	-	
	KZGN11O139.CN0182	1995	GD	Water	1	-	-	-	
	KZGN11O139.CN0051	2006-2007	JX	Water	7	-	-	-	
	KZGN11O139.CN0010	2005	JX	Water	2	-	-	-	
	KZGN11O139.CN0091	2006	GD	Water	1	-	-	-	
	KZGN11O139.CN0227	2007	JX	Water	1	-	-	-	
	KZGN11O139.CN0050	2006-2007,2009	GD,JX	Water	11	-	-	-	
	KZGN11O139.CN0209	2007-2008	JX	Water	2	-	-	-	
	KZGN11O139.CN0229	2008	JX	Water	1	-	-	-	
	KZGN11O139.CN0181	2006	FJ	Water	1	-	-	-	
	KZGN11O139.CN0087	2006	GD	Water	1	-	-	-	
	KZGN11O139.CN0088	2006	GD	Water	1	-	-	-	
	KZGN11O139.CN0328	2010	GD	Water	1	-	-	-	
	KZGN11O139.CN0329	2010	GD	Water	1	-	-	-	
	KZGN11O139.CN0022	1995	FJ	Patient	1	+	+	+	
	KZGN11O139.CN0093	2005	SD	Patient	1	+	+	+	
	KZGN11O139.CN0109	1998	JS	Patient	1	+	+	+	
	KZGN11O139.CN0204	1994	GD	Patient	1	+	+	+	
	KZGN11O139.CN0006	1998,2000,2002,2004	AH,JX	Patient,Water	4	+	+	+	
	KZGN11O139.CN0019	1994-1995	BJ,GD	Patient	2	+	+	+	
	KZGN11O139.CN0019	1994	JX	Patient	1	-	-	-	
	KZGN11O139.CN0198	1994	SH	Patient	1	+	+	+	
	KZGN11O139.CN0237	2010	LN	Patient	1	+	+	+	
	KZGN11O139.CN0150	1997	LN	Patient	1	+	+	+	
	KZGN11O139.CN0076	1996	ZJ	Patient	1	+	+	+	
	KZGN11O139.CN0145	2003	ZJ	Patient	1	+	+	+	
	KZGN11O139.CN0005	2003-2007	CQ,JS,JX,SC,ZJ	Patient,Water,Soft-shelled turtle	11	+	+	+	
	KZGN11O139.CN0005	2005	JS	Sample of food poisoning	1	-	-	-	
	KZGN11O139.CN0100	2006	GD	Patient	1	+	+	+	
	KZGN11O139.CN0219	2007	JX	Soft-shelled turtle	1	+	+	+	
	KZGN11O139.CN0148	2002	CQ	Patient	1	+	+	+	
	KZGN11O139.CN0074	1999	JS	Patient	1	+	+	+	
	KZGN11O139.CN0149	2001	LN	Patient	1	+	+	+	
	KZGN11O139.CN0077	1999,2002	GD,JX	Patient	5	+	+	+	
	KZGN11O139.CN0158	2007	SC	Patient,Water	2	+	+	+	
	KZGN11O139.CN0196	1997	ZJ	Patient	1	+	+	+	
	KZGN11O139.CN0151	2006	CQ,JS	Patient	2	+	+	+	
	KZGN11O139.CN0052	2006	GD	Water	2	+	+	+	
	KZGN11O139.CN0154	2002	GS	Patient	1	+	+	+	
	KZGN11O139.CN0081	2006	SC	Patient	1	+	+	+	
	KZGN11O139.CN0002	2007	CQ,FJ,GD,HN,JS,JX,SC,SD	Patient,Water,Soft-shelled turtle	25	+	+	+	
	KZGN11O139.CN0001	2003,2008-2009	JX,SD	Patient,Water	5	+	+	+	
	KZGN11O139.CN0004	1998,2000-2001	GX,JX	Patient,Water	6	+	+	+	
	KZGN11O139.CN0155	2005	SH	Soft-shelled turtle	1	+	+	+	
	KZGN11O139.CN0078	2006	GD	Patient	1	+	+	+	
	KZGN11O139.CN0143	1994	ZJ	Patient	1	+	+	+	
	KZGN11O139.CN0221	2007	JX	Water	1	+	+	+	A
	KZGN11O139.CN0082	2005	ZJ	Water	1	+	+	+	
	KZGN11O139.CN0068	2003	JS	Patient	1	+	+	+	
	KZGN11O139.CN0157	2005	SH	Soft-shelled turtle	1	+	+	+	
	KZGN11O139.CN0104	2002	JX	Water	1	+	-	-	
	KZGN11O139.CN0047	2006,2008	GX,JS,JX,SC	Patient,Water,Bullfrog,Soft-shelled turtle	10	+	+	+	
	KZGN11O139.CN0069	2000	JX	Patient	1	+	+	+	
	KZGN11O139.CN0046	2003,2006	GD,JX	Patient,Water	3	+	+	+	
	KZGN11O139.CN0056	2006	SC	Water	1	+	+	+	
	KZGN11O139.CN0160	2000	GX,JX	Patient	2	+	+	+	
	KZGN11O139.CN0326	2002	GD	Patient	1	+	+	+	
	KZGN11O139.CN0142	1993	XJ	Patient	1	+	+	+	
	KZGN11O139.CN0066	2007	AH,JX	Patient,Water	3	+	+	+	
	KZGN11O139.CN0161	2005	GX	Water	1	+	+	+	
	KZGN11O139.CN0201	2005	GD	Patient	1	+	+	+	
	KZGN11O139.CN0011	2005	HN	Fish	1	+	+	+	
	KZGN11O139.CN0063	2003	JS	Patient	1	+	+	+	
	KZGN11O139.CN0107	2007	GD	Water	1	+	+	+	
	KZGN11O139.CN0236	2010	LN	Patient	1	+	+	+	
	KZGN11O139.CN0172	2006	AH	Patient	1	+	+	+	
	KZGN11O139.CN0116	2002	JX	Patient	1	+	+	+	
	KZGN11O139.CN0152	2001	SC	Patient	1	+	+	+	
	KZGN11O139.CN0122	1995	GD	Patient	1	+	+	+	
	KZGN11O139.CN0187	1993	XJ	Patient	1	+	+	+	
	KZGN11O139.CN0314	1996	GD	Patient	1	+	+	+	
	KZGN11O139.CN0086	1993	XJ	Patient	1	+	+	+	
	KZGN11O139.CN0012	2001	GD	Patient	1	+	+	+	
	KZGN11O139.CN0073	2006,2009	HA,YN	Patient,Soft-shelled turtle	6	+	+	+	
	KZGN11O139.CN0045	2003	CQ	Patient	1	+	+	+	
	KZGN11O139.CN0014	2005	CQ	Soft-shelled turtle	1	-	-	+	
	KZGN11O139.CN0162	2007	SC	Patient,Water	2	+	+	+	
	KZGN11O139.CN0300	2012	JS	Mutton	1	+	+	+	
	KZGN11O139.CN0133	2006	GD	Water	1	-	-	-	
	KZGN11O139.CN0140	2004	LN	Patient	1	-	-	-	
	KZGN11O139.CN0016	2005	BJ	Patient	1	-	-	-	
	KZGN11O139.CN0049	2006	JX	Water	2	-	-	-	
	KZGN11O139.CN0164	2006	GX	Water	1	-	-	-	
	KZGN11O139.CN0131	2001	JX	Water	1	-	-	-	C
	KZGN11O139.CN0132	1999	JX	Water	1	-	-	-	
	KZGN11O139.CN0130	2000	JX	Water	1	-	-	-	
	KZGN11O139.CN0327	2009	GZ	Soft-shelled turtle	1	-	-	-	
	KZGN11O139.CN0225	2008	JX	Water	1	-	-	-	

PT235 The glutamine-depend acid resistance system in neutralophilic bacteria

Eugenia Pennacchietti[1], Chiara D'Alonzo[1], Luca Freddi[2], Alessandra Occhialini[2], Daniela De Biase[1]

[1]*Sapienza Univeristy of Rome, Dpt of medico-surgical Sciences and Biotechnologies, Latina, Italy*
[2]*Institut de Recherche en Infectiologie de Montpellier (IRIM), UMR 9004 CNRS, Montpellier, France*

Background: The amino acid-dependent acid resistance (AR) systems are widespread in neutralophilic bacteria. Their activities overlap so to cover a rather large acidic pH range, from 6 to <2. Recently, the AR system relying on glutamine (AR2_Q) was shown to be operative in *Escherichia coli*, *Lactobacillus reuteri* and some *Brucella* species. AR2_Q requires the glutaminase GlsA/YbaS and the membrane antiporter GadC, both proteins are active at acidic pH. GadC imports *L*-glutamine and exports either glutamate (the glutamine deamination product) or GABA, the decarboxylation product of glutamate, via glutamate decarboxylase (GadB), a structural component of the glutamate-dependent AR (AR2) system, together with GadC.

Objectives: This work was undertaken to study the distribution of the AR2_Q system in bacteria and to develop a qualitative acid-glutaminase assay and a quantitative GadC-mediated transport assay.

Methods: The qualitative assay takes advantage of the color change of the pH indicator bromocresol green. The quantitative assay is fluorometric, HPLC-based for detection of extracellular glutamine, glutamate and GABA.

Results: The colorimetric assay is rapid and reliable. It also provides information on co-occurrence of AR2 and AR2_Q in a bacterial species. The HPLC-based assay allows to measure the uptake of glutamine and the export of glutamate and/or GABA via GadC in the pH range 2.5-4.0. A bioinformatic genome analysis showed that the gene encoding the acid glutaminase is often nearby or in operon arrangement with the genes encoding GadC and GadB. Overall, our results indicate that AR2_Q is likely to be of prominent importance especially for enteric bacteria AR.

PT236 A new class of base-activating enzymes involved in cobamide biosynthesis in methanogenic archaea and cyanobacteria

Victoria L. Jeter[1], Theodoric A. Mattes[1], Nathaniel R. Beattie[2], Jorge Escalante-Semerena[1]

[1]*University of Georgia, Microbiology, Athens, United States*
[2]*University of Georgia, Biochemistry & Molecular Biology, Athens, United States*

Background: Cobamides (Cbas) are coenzymes used by cells from all domains of life. The last steps of the cobamide biosynthetic pathway activate the corrin ring and the lower ligand base, condense these intermediates and dephosphorylate the product yielding the coenzyme. Activation of the base is catalyzed by a nicotinate mononucleotide(NaMN): 5,6-dimethylbenzimidazole(DMB) phosphoribosyltransferase (CobT) generating the intermediate α-ribazole-phosphate. The crystal structure of NaMN:DMB phosphoribosyltransferase from *Methanocaldococcus jannaschii* (PDB 3L0Z) has been reported, and comparisons to *Salmonella enterica* CobT (*Se*CobT) show limited identity and similarity. Here we report the biochemical and functional characterization of *M. jannaschii* CobT (*Mj*CobT).

Objectives: To characterize a new class of NaMN:DMB phosphoribosyltransferases found in methanogenic archaea and cyanobacteria.

Methods: NaMN:DMB phosphoribosyltransferase activity of *Mj*CobT was shown *in vivo* using a *S. enterica* Δ*cobT* strain. Substrate specificity was assessed by HPLC/MS analysis of extracted cobamides. *Mj*CobT was purified and its activity optimized *in vitro*. NaMN substrate affinity was analyzed by isothermal titration calorimetry and oligomeric state was determined by analytical ultracentrifugation. *Mj*CobT homologue *Synechococcus* sp. WH7803 CobT phosphoribosyltransferase activity was shown *in vivo*.

Results: *In vivo* and *in vitro* data reported here confirm that *Mj*CobT is a new class of NaMN:DMB phosphoribosyltransferases common to methanogenic archaea. Our results show this class of CobT can activate adenine and benzimidazole bases similarly to *Se*CobT, but with a highly divergent structure. Additionally, our studies show that most cyanobacterial genomes encode *Mj*CobT homologues. In support of this conclusion, we showed the cyanobacterial *Synechococcus* sp. CobT was a functional enzyme *in vivo.*

PT237 Structural studies on a bacterial membrane protein important for survival

Andreas Kiessling[1]

[1]*University of Leeds, Faculty of Biological Sciences, Leeds, United Kingdom*

Background: The efflux transporter AceI from *Acinetobacter baumannii* confers resistance to the bactericide chlorhexidine. The mechanism of transport is still unknown and no structure of this protein has been solved yet. This study allows an insight into the oligomeric composition of AceI and gives a good estimate on future applicability of cryo-EM for structure determination for these kind of small membrane proteins.

Objectives: This work aims to provide low-resolution structural data on AceI in its natural lipid environment via extraction and purification in a styrene-maleic acid (SMA) polymer and subsequent analysis with negative stain electron microscopy (NS-EM).

Methods: AceI with a C-terminal-StrepII-tag was expressed in *E. coli* C43(DE3) using the pTTQ18 vector and solubilised with n-Decyl-β-D-Maltopyranoside (DM), n-Dodecyl-β-D-maltoside (DDM) and SMA polymer. It was then purified over a Streptactin resin followed by size-exclusion chromatography (SEC). I then stained with 1 % uranyl acetate to assess the heterogeneity and composition of the protein sample via NS-EM.

Results: Western blot confirmed that AceI is tetrameric in SMA. Initial 2D classes from NS-EM revealed four individual monomers tightly packed in a cubic form which has an approximate size of <100 $Å^3$. The data suggest that EM is unlikely to be able to solve the structure, as few of the 2D classes could be safely identified as tetrameric. This information was used to create an *in silico* 3D model of the closed state with the key conserved F94 in a positively charged pocket on the cytoplasmic side of the protein.

PT238 Complete genome assessment targeting risk factors of Lactobacillus reuteri PNW1 - an interesting probiotic candidate

Kazeem Adekunle Alayande[1;2], Collins Njie Ateba [1], Olayinka Ayobami Aiyegoro [2]

[1]*North-West University, Microbiology and Botany, Mahikeng, South Africa*
[2]*Agricultural Research Council - Animal Production Institute, Gastrointestinal Microbiology and Biotechnology Division, Pretoria, South Africa*

Background: Although most probiotic strains have acquired the "Generally Recognized As Safe" status, notwithstanding the clinical efficacy of probiotics, every novel strain cannot be assumed to share the historical safety with conventional strains. Just as it occurs in other organisms, probiotics may possess undesirable properties.

Objectives: Complete genome of the isolate was analysized for possible presence of virulence determinant, antimicrobial resistance, haemolytic potential and production of toxic biochemicals.

Methods: The bacterium was isolated and characterized from the gastro-intestinal tracts of compassionately sacrificed weaned piglets of indigenous South African Windsnyer pig breed. Its genomic DNA was extracted with DNA extraction kit (zymo research, USA). The genome was prepared using Illumina NGS Library prep kit, and run on Illumina MiSeq technology platform1. The data was filtered for low quality reads and adapter regions using Trimmomatic version 0.32. A draft genome assembly was constructed using SPAdes version 3.7.1. Genome annotation was carried out by NCBI Prokaryotic Genome Annotation Pipeline (PGAP) and Rapid Annotations using Subsystems Technology (RAST) (SRA accession: PRJNA504734). Pathogenicity towards human host was predicted using PathogenFinder.

Results: A total of 8,209,104 paired-end reads were generated. The assembled genome is 2,430,215 bp long in 420 contigs. The N_{50} is 28,048 bp and average G+C content is 39%. The number of protein coding sequences and structural RNAs are 2581 and 79 respectively. The isolate is predicted as non human pathogen. No remarkable virulence and biogenic amine associated genes detected. The genome analysis only indicates presence of Tetracyclinc (*TetW*) and Lincosamide (*InuC*) resistant genes.

PT239 Dormant and metabolic-active persister formation in Escherichia coli mediated by the expression of lactate dehydrogenase A

Naoki Yamamoto[1], Yurino Ohno[1], Rino Isshiki[1], Yuto Kawai[1], Shinya Matsumoto[2], Satoshi Tsuneda[1]

[1]*Waseda University, Life Science and Medical Bioscience, Tokyo, Japan*
[2]*Nagoya University, Molecular Bacteriology, Nagoya, Japan*

Background: In recent years, the emergence of bacterial persister is a cause to fail pathogenic infectious disease. Persister is subpopulation that is dormant, tolerant against antibiotics, and resuscitate after antibiotic removal. Though the multiple mechanisms underlying persister formation are reported, most of them remain unknown and general understanding of persister is required to completely cure pathogenic infection.

Objectives: We previously performed comparative microarray analysis between dividing cells and non-dividing persister cells, and then we found that lactate dehydrogenase A gene (*ldhA*) expression induced *E. coli* persister formation by measuring survival rate and analyzing single-cell behavior. In this study, we aim to understand the detailed mechanism of *ldhA*-mediated persister formation.

Methods: To investigate the relationship between *ldhA* expression and energy metabolism, we determined the proton motive force (PMF) and ATP level. Moreover, we investigated how *ldhA*-induced metabolic change is related to antibiotic survival through measurement of *recA* expression level by qRT-PCR.

Results: Lactate dehydrogenase pathway is known to compete aerobic metabolism pathway for pyruvate, and it is reported that the repression of aerobic metabolism such as PMF and ATP synthesis induced persister. Therefore, we hypothesize that *ldhA* expression induces the previously reported persister mechanism through repressing aerobic metabolism. Unexpectedly, we found that *ldhA* expression increased PMF and ATP level. Moreover, we found that *ldhA* induced the expression level of *recA*, ATP-dependent DNA repair system. These findings indicate that accumulated energy by *ldhA* expression distributes various stress response such as DNA repair, and thereby antibiotic tolerance is induced in *E. coli*.

PT240 Functional analysis of the mpsABC cluster in Staphylococcus aureus

Sook-Ha Fan[1], Patrick Ebner[1], Sebastian Reichert[1], Friedrich Goetz[1]

[1]*Interfaculty Institute for Microbiology and Infection Medicine Tübingen (IMIT), Microbial Genetics, Tuebingen, Germany*

Background: We previously reported that the deletion of *mpsABC* (mps for membrane potential-generating system) in *Staphylococcus aureus* resulted in an impairment in membrane potential generation of the cells cultivated in aerobic conditions. MpsABC was demonstrated to have cation-translocating system capability and responsible for the generation of membrane potential. MpsA identified in *S. aureus* shows sequence similarity to the proton- translocating subunit NuoL of complex 1 in *E. coli*. *mpsA* is the first part of an operon comprising three genes: *mpsA, mpsB* and *mpsC.* MpsB and MpsC show no significant sequence homologies to proteins with known function.

Objectives: Deletion mutant of *mpsABC* was found to be severely affected in growth which mostly could be attributed to its decreased membrane potential. This study aims to investigate the various conditions and supplementations that could rescue the disruption in membrane potential generation.

Methods: The growth of the deletion mutant was tested in high CO_2 and different pH conditions as well supplementation with various co-factors and TCA cycle substrates. Any significant growth-promoting effect seen was further checked for the restoration of membrane potential.

Results: In the presence of high CO_2 conditions, growth of *ΔmpsABC* and its membrane potential was restored to wild-type level. With regard to these observation, the *mpsABC* cluster is hypothesized to have an extensive role in growth and possibly CO_2 assimilation.

PT241 BfmR regulator from Acinetobacter baumannii modulates Hcp secretion without effecting killing phenotype mediated by type VI secretion system

Renatas Krasauskas[1], Jūratė Skerniškytė[1], Julija Armalytė[1], Edita Sužiedėlienė[1]

[1]*Institute of Biosciences, Life Sciences Center, Vilnius University, Vilnius, Lithuania*

Background: In recent years, *Acinetobacter baumannii* has become one of the most important Gram-negative opportunistic pathogen responsible for hospital-acquired infections. Studies have shown that *A. baumannii* two component signal transduction system BfmRS, consisting of response regulator BfmR and sensor kinase BfmS, is implicated in the control of virulence-related traits such as resistance to complement and desiccation stress, and has been suggested to act as a global modulator of *A. baumannii* physiology in infection.

Objectives: To assess the role of BfmRS in *A. baumannii* type VI secretion system (T6SS) regulation.

Methods: Generated *ΔbfmRS* and/or *Δhcp* mutants of *A. baumannii* clinical strain V15 were complemented with wild-type or inactive *bmfR* alleles and various clinically important phenotypic assays as well as interbacterial competition with *E. coli* MC4100 were performed.

Results: We showed that the loss of *ΔbfmRS* results in greatly reduced secretion of *A. baumannii* T6SS component – Hcp protein. Also, we observed that *ΔbfmRS* mutants displayed transcriptional down-regulation of T6SS system. Remarkably, the reduction of extensive Hcp secretion was not associated with the down-regulation of T6SS-mediated killing phenotype against *E. coli* strain MC4100. In addition, Hcp was not required for the formation of biofilms. The observed phenotype in *ΔbfmRS* mutant was restored by the introduction of wild-type *bfmR* allele, confirming that Hcp secretion was BfmR-dependent. In conclusion, our results suggest that Hcp might play additional, BfmR-regulated, role in *A. baumannii* physiology.

PT242 Molecular characterization of a dual activity metal and antibiotic efflux pump from Mycobacterium tuberculosis

Anwesha Adhikary[1], Anindya Sundar Ghosh[1]

[1]*Indian Institute of Technology Kharagpur, Biotechnology, Kharagpur, India*

Background: "Multi-drug-resistant" (MDR) pathogens are causing a havoc on antimicrobial therapy. Among the several mechanisms of resistance, effflux pumps are the options where bacteria can develop resistance against several structurally unrelated antibiotics. Tolerance to high concentration of metals and antibiotics would be threatening if the mechanisms are linked. Though, the evidences are scanty in favour of such dual activity efflux pumps.

Objective: To understand the antibiotic export ability of a putative metal efflux pump protein of *Mycobacterium tuberculosis* that belongs to the NiCoT family of transporters.

Methods: The Rv2856/nicT gene was cloned in pBAD18-cam and ectopically expressed in *E. coli*. Minimum inhibitory concentration (MIC) of different antibiotics, reagents and metal salts against the strain was determined. Change in the MIC was tested in the presence of subinhibitory concentration of metal ions. Antibiotic accumulation in the cells over time was measured by fluorimetric assay.

Results: The ectopic expression of the gene Rv2856/nicT in *E. coli* results in the enhanced resistance towards Gentamicin, Norfloxacin, Ofloxacin, Amikacin and Nalidixic acid. Accumulation assay of Norfloxacin also corroborated this findings. Moreover, in the presence of Ni^{2+}ions, an increase in the resistance towards Kanamycin and Tetracycline is observed. Similar results are also observed for three other putative efflux pumps, namely, Rv1877, Rv0676c, Rv1273c of *Mycobacterium tuberculosis*. Our study indicates a dual resistance trait of the aforementioned efflux pump protein where metals might play an integral role in the antibiotic resistance development, though the exact mechanism is so far unknown.

PT243 Residues of the corrinoid adenosyltransferase EutT from L. monocytogenes involved in ATP binding and corrinoid reduction

Flavia Costa[1], Jorge Escalante-Semerena[1]

[1]*University of Georgia, Athens, United States*

Background: ATP:Co(I)rrinoid adenosyltransferases (ACATs) catalyze the formation of the unique C-Co bond of adenosylcobalamin. There are three types of ACATs: CobA, PduO, and EutT. Class I EutTs require a metal, whereas Class II EutTs are metal-less. Both EutT classes have been studied to some extent, however the ATP and corrinoid binding sites and residues involved in the reduction of Co(II) cobalamin to Co(I) cobalamin of Class II EutTs are unknown.

Objectives: Determine how the Class II EutT-type ACAT *from Listeria monocytogenes* (*Lm*EutT) binds Mg/ATP and reduces Co(II) cobalamin to Co(I) cobalamin prior to catalysis.

Methods: Analysis of the amino acid sequence of *Lm*EutT revealed six conserved residues corresponding to the ATP-binding site of *Lactobacillus reuteri* PduO (*Lr*PduO). Kinetic analysis of *Lm*EutT variants with substitutions at putative ATP-binding residues revealed two variants that lacked enzymatic activity and four variants whose $K_{0.5}$ increased by 4 to 100-fold.

In the CobA ACAT of *Salmonella enterica* and *Lr*PduO*,* bulky hydrophobic side chains facilitate the reduction of Co(II) cobalamin. The *Lm*EutT (LmEut^{F72A}) variant displayed decreased reduction of Co(II) cobalamin, and its turnover rate was increased when the reaction contained Co(I) cobalamin. These results suggest that residue F72 is involved, but not critical, in facilitating the reduction of Co(II) cobalamin. Work is ongoing to understand the role of this residue in cobalamin reduction.

Results: We suggest that PduO and EutT-type ACATs share an ATP-binding site, which may inform the evolutionary origin of these enzymes. The mechanism of cobalamin reduction of metal-less EutTs is unique among ACATs.

PT244 Differentiation of bacterial species and methicillin-resistant Staphylococcus aureus by consumption profile of nine amino acids

Chi-Chung Chou[1], Yung-Chun Chang[1], Chi-Hsuan Sung [1]

[1]National Chung-Hsing University, Veterinary Medicine, Taichung, Taiwan

Background: Bacterial differentiation can be done by many approaches but none rely solely on the organism's ability to utilize amino acids (AAs). Bacteria may comsume AAs differently depending on their genetic traits and biochemical requirements. It is hypothesized that intrinsic AA utilization profiles could be an alternative tool for differentiation of bacteria at different applications.

Objectives: The study was designed to profile the bacterial use of 20 common essential and non-essential amino acids as a novel tool for classifying of bacterial species and assessment of resistance to Methicillin.

Methods: More than 170 strains of *Escherichia coli, Staphylococcus hyicus, Enterococcus faecalis, Salmonella enterica, Pseudomonas aeruginosa, Pasteurella multocida,* and *Staphylococcus aureus* (both methicillin-sensitive and resistant) were analyzed and compared for their ability to utilize 20 AAs. The bacteria were cultured in minimum-nutritional media containing 3 mM of single AA for 18 hours and each AA consumption was analysed by a HPLC system coupling with flow-injection analysis using electrochemical detection (FIA-EC).

Results: While similarities do exist, the 7 bacterial species each presented a charastistic pattern utiling the 20 AAs. More than 98% of the test strains can be classified by using profiling of only 9 AAs including Glu, Gly, Lys and Ser. Eight AAs including Val and Leu showed striking differences in their utilization by MRSA strains. The tested bacteria utilize AAs in a species-dependent manner such that cumsumption profiles of specific AAs are characteristic enough to afford a novel tool for bacterial classification and typing of methicillin-resistant strains.

PT245 K224A substitution in the loop near to active site of Oxa-23 drastically affects its beta-lactamase activity

Diamond Jain[1], Anindya Sundar Ghosh[1]

[1]*Indian Institute of Technology, Department of Biotechnology, Kharagpur, India*

Background: Carbepenem group of antibiotics are widely used to treat infections caused by multi-drug resistant (MDR) bacteria. Evolution of carbapenemases has developed intrinsic resistance in MDR strains leading to a situation that is critical for antimicrobial therapy. It has been reported that amino acids in the non-active site might affect the beta-lactam hydrolyzing ability of carbapenemases.

Objectives: To elucidate the role of amino acid residue(s) other than the active-site of Oxa-23 in gram negative bacteria.

Methods: *bla*OXA-23 gene was detected from clinical isolates and further cloned in pBAD18cm vector for *in-vivo* studies. Mutants were created using site directed mutagenesis method. *In-vivo* efficiency of Oxa-23 and its mutant were analyzed in *E.coli* CS109 by determining the change in the antibiotic resistance. Truncated forms (devoid of signal peptide) of both *bla*OXA-23 and its mutants were cloned in pET28a vector for cytoplasmic expression and purification using Ni^{2+} 6X-His-tag affinity chromatography. The *in-vitro* enzymatic efficiencies of OXA-23 and its mutants were analysed by checking the nitrocefin hydrolytic rates of the enzymes.

Results: Among D222A, K224A and Q226A mutational substitutions in β5-β6 loop of Oxa-23, K224A had a drastic effect on *in-vivo* efficiency of Oxa-23, leading to several fold reductions in MIC values. CS109 cells harbouring Oxa-23_K224A showed least efficiency in resisting the penicillin, cephalosporin and carbepenem groups of antibiotics. Therefore, it is concluded that substitution of K224A in Oxa-23 has drastically reduced the enzymatic efficiency, both in vivo and in vitro and the information may be helpful in designing future inhibitors against Oxa-23.

PT246 Changes in the metabolic fluxes of Pseudomonas putida KT2440 during its exponential growth in a complete medium derived from the sequential consumption of the carbon sources

Lázaro Molina[1], Ruggero La Rosa[2], Juan Nogales[3], Fernando Rojo[4]

[1]Centro Nacional de Biotecnología, CSIC, Microbial Biotechnology, Madrid, Spain
[2]Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark, Kongens Lyngby, Denmark
[3]Centro Nacional de Biotecnología, CSIC, Systems Biology, Madrid, Spain
[1]Centro Nacional de Biotecnología, CSIC, Microbial Biotechnology, Madrid, Spain

Background: When *Pseudomonas putida* is cultured in a rich medium, carbon sources are used in a hierarchical and sequential manner. This implies important changes in the gene expression programs and in metabolism. Although the molecular details underlying this regulatory response have been investigated for decades, the final effect on the metabolic fluxes is poorly known.

Objectives: Our aim was to analyse the changes occurring in *P. putida* KT2440 metabolic fluxes while it grows exponentially in LB medium and sequentially assimilates the compounds available.

Methods: To determine the metabolic rearrangements that occurred throughout exponential growth in LB, the assimilation rates for >35 compounds of LB were determined at three different moments during the exponential growth. These data were integrated with the changes observed in the proteome, and with the metabolic fluxes predicted by the *i*JN1411 metabolic model for this strain at these same time points.

Results: The results indicate the bacterium to use the different components sequentially, although several were simultaneously assimilated at any given moment. Sugars were consumed differently during the early, mid and late exponential phases of growth, the fluxes through the tricarboxylic acid cycle varying between these times. The cells thus adapted to the availability of the compounds present without interrupting growth. This highlights the dynamism and flexibility of *P. putida* metabolism.

Detailed knowledge of the metabolic fluxes that operate under different growth conditions is essential if we are to understand how cells assimilate compounds in the environment, and are to manipulate their metabolism for biotechnological purposes.

PT247 An auxin as a signal that regulates antibiotic production in a rhizospheric bacterium

Miguel A. Matilla[1], Abdelali Daddaoua[2], Andrea Chini[3], Bertrand Morel[4], Tino Krell[2]

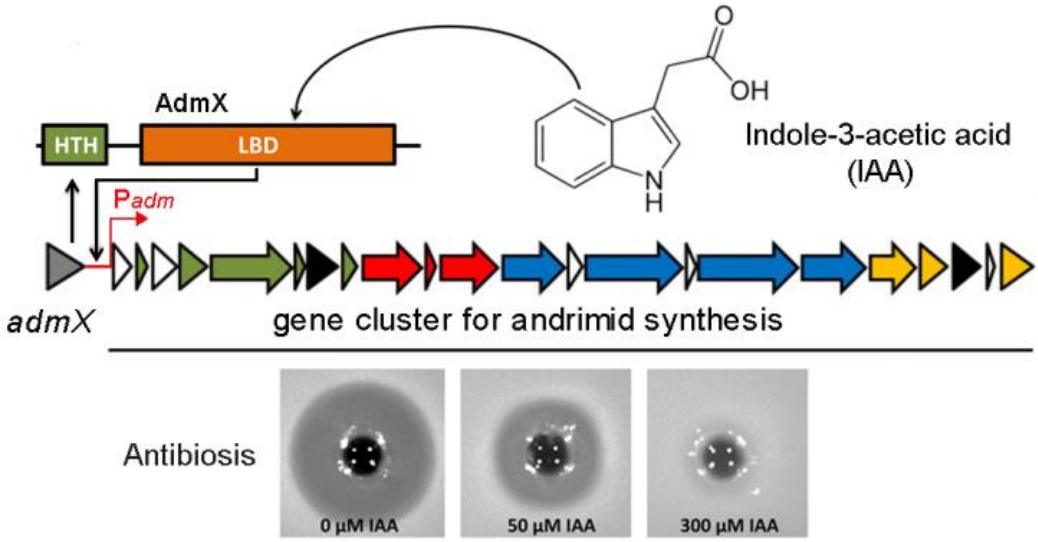
[1]*Estación Experimental del Zaidín, Environmental Protection, Granada, Spain*
[2]*Estación Experimental del Zaidin, Environmental Protection, Granada, Spain*
[3]*National Centre for Biotechnology, Plant Molecular Genetics, Madrid, Spain*
[4]*University of Granada, Physical Chemistry and Institute for Biotechnology, Granada, Spain*

Background: Microbes are a prolific source of bioactive secondary metabolites and great efforts are being made in order to isolate new antibiotics that counteract the emergence of multidrug-resistant microorganisms. The production of these metabolites is highly regulated and many antibiotics are cryptic, what hinders the identification of novel bioactive metabolites. The rhizobacterium *Serratia plymuthica* A153 produces a broad range of antibiotics, including the bacterial acetyl-CoA carboxylase inhibitor, andrimid. The synthesis of this hybrid polyketide-nonribosomal peptide is activated by the pathway-specific LysR-type transcriptional regulator, AdmX. Based on the molecular mechanism of LysR regulators, it was hypothesized that specific signals control AdmX function.

Objectives: We aimed at identifying signalling molecules that control andrimid production through the modulation of AdmX activity.

Methods: AdmX was purified and used for high-throughput ligand screenings, hits were verified by microcalorimetry and the mechanism of action deciphered by *in vivo* and *in vitro* transcription assays, EMSA and antibacterial assays.

Results: We found that the auxin indole-3-acetic acid (IAA) specifically binds to the ligand binding domain of AdmX. This binding causes conformational changes in AdmX that result in the inhibition of transcription of the andrimid biosynthetic cluster and in the suppresion of antibiotic production in a dose-dependent manner. Our results also show that IAA synthesis by pathogenic and beneficial plant-associated bacteria inhibits andrimid production in A153. Take together, this study unravels the molecular mechanism by which IAA modulates andrimid synthesis in rhizospheric bacteria and highlights the relevance of this auxin in intra- and inter-kingdom interactions that regulate antibiotic production.


AdmX
HTH
LBD
Padm
admX
gene cluster for andrimid synthesis
O
OH
N
H
Indole-3-acetic acid
(IAA)

Antibiosis
0 µM IAA
50 µM IAA
300 µM IAA

PT248 Post-Antibiotic Effect of Moxifloxacin in Streptococcus pneumoniae: DNA-cleavage and Reactive Oxygen Species Production

Maria Teresa Garcia Esteban[1], Myriam Valenzuela[1], María José Ferrándiz[2], Adela G. de la Campa[2]

[1] Universidad Complutense, Facultad de C.C. Biológicas, Genética, Fisiología y Microbiología, MADRID, Spain
[2]Instituto de Salud Carlos III, Centro Nacional de Microbiología, Unidad de Genética Bacteriana, Majadahonda, Madrid, Spain

Background: *Streptococcus pneumoniae* is cause of community acquired pneumonia, meningitis, otitis media, sinusitis and conjunctivitis. Moxifloxacin (MFX) is a fluoroquinolone used for pneumococcal infections. The post-antibiotic effect (PAE) is useful to determine optimal dosage regimens for antibiotics. MFX forms intermediates of DNA-MFX-topoisomerase complexes, which generates double-stranded DNA breaks. Its lethality is enhanced by the production of reactive oxygen species (ROS), via the up-regulation of genes that increases pyruvate and stimulate the Fenton reaction.

Objectives: To study the factors involved in PAE of MFX in *S. pneumoniae*

Methods: *Streptococcus pneumoniae* R6 strain was analyzed during the postantibiotic phase. Growth curves by viable counting, level of chromosome fragmentation by Pulse Field Gel Electrophoresis, ROS production by dihydrorhodamine 123 dye and gene expression by q-RT-PCR, were determined

Results: Treatment of 1 h with MFX at (2.5 –5 – 10) × MIC induced PAE of 0.76 h, 1.33 h and 1.99 h, respectively. DNA breakage induced by the treatment decreased more than 3-fold after the PAE-phase. Increase up to 3-fold of ROS level induced by the treatment reverted after the PAE-phase. MFX up-regulated the expression of *fba and gpdA* genes involved in the conversion of fructose 6-phosphate to pyruvate by glycolytic pathway. MFX also up-regulated the expression of *pmi* and *tktA* genes, which encode for enzymes that funnel phosphate sugars into the glycolytic pathway. MFX induces significant PAE representing the time necessary for the bacteria to recover their normal levels of ROS and intact DNA.

PT249 A novel lactobacillus rhamnosus gg-derived protein exerts anti-inflammation through nf-κb inactivation

Yubin Li[1], Xiaolong He[1], Yu Wan[1], Zelong Gong[1], Hong Cao[1]

[1]*Department of Microbiology, School of Public Health, Southern Medical University*

Background: Postbiotics has been implicated for the treatment of intestinal dysfunction. In the recent research, p40 was confirmed to prevent cytokine-induced apoptosis and intestinal dysfunction in colon, while the other functional components and specific mechanism are not clear yet. Postbiotics has been the hot spot in treatment of inflammatory bowel disease.

Objectives: Our study explore whether protein HM0539 suppress inflammatory response in LPS - induced RAW 264.7 cells and DSS-induced mice colitis through inhibition of NF-κB signaling pathway.

Methods: The effects of HM0539 were evaluated by nitric oxide (NO) production and expression of inducible NO synthase (iNOS) and nuclear factor-kappa B (NF-κB) in LPS-induced RAW264.7 via the Griess assay, Western blotting. The secretion of cytokine (IL-6, IL-1β, TNF-α, prostaglandin E2) were determined via enzyme-linked immunosorbent assay. Ulcerative colitis mice model induced by 3% (w/v) dextran sodium sulfate was used to assess the effect of HM0539.

Results: Western blot analyses revealed that HM0539 suppressed the protein expression of NF-κB p65, p-NF-κB p65, iNOS and COX-2, which subsequently reduced expression of NO and PGE2. It also significantly inhibited the production of cytokine, such as IL-1β, TNF-α. Moreover, HM0539 ameliorates DSS-induced mice colitis indicated by reduced colitis symptoms including disease activity index, body weight loss, colon length, colonic mucosal macroscopic damage and hematochezia score. Taken together, HM0539 might play a potential therapeutic agent for inflammation by suppressing the activation of the NF-κB signaling pathways. (*Corresponding author: Hong Cao, E-mail: gzhcao@smu.edu.cn)

PT250 The structure and biosynthesis gene cluster of a novel glycopeptidolipid isolated from clinical nontuberculous mycobacteria

Nagatoshi Fujiwara[1], Yuji Miyamoto[2], Minoru Ayata[3], Takashi Naka[1], Hirotaka Kuwata[4], Hiroyuki Yamada[5], Shinji Maeda[6]

[1]Tezukayama University, Faculty of Contemporary Human Life Sciences, Nara, Japan
[2]National Institute of Infectious Diseases, Leprosy Research Center, Japan
[3]Osaka City University Graduate School of Medicine, Department of Virology, Japan
[4]School of Dentistry, Showa University, Department of Oral Microbiology and Immunology
[5]Research Institute of Tuberculosis, JATA, Japan
[6]Hokkaido University of Science, Department of Pharmaceutical Sciences, Japan

Background: *Mycobacterium avium* and *Mycobacterium intracellulare* (MAC) are the most common isolates of nontuberculous mycobacteria, which cause pulmonary diseases. MAC species are classifiable into 28 serotypes based on the epitopic oligosaccharide structure of the serotype-specific glycopeptidolipid (GPL) antigen. We checked the heterogeneity of serotype-specific GPLs in clinical isolates. In the process, *M. intracellulare* Ku11 strain was isolated from a patient in Japan. This strain produced a novel GPL, which showed a different Rf value on thin-layer chromatography (TLC) from those of other standard GPLs.

Objectives: We clarify the novel structure and biosynthesis gene of Ku11-GPL in clinical isolates, and discuss the structure-function relationships.

Methods: The oligosaccharide of Ku11-GPL was analyzed by using chromatography, mass spectrometry (MALDI/TOF-MS and GC/MS) and NMR. The gene cluster involved in GPL biosynthesis was isolated and sequenced. We checked the similarity of the deduced amino acid sequences in the open reading frames (ORFs). *M. smegmatis* produced non-specific apolar GPL. We tried to introduce some ORFs into the *M. smegmatis* strain which was recombinantly modified to produce serotype 1-GPL.

Results: The sugar moiety of Ku11-GPL was defined as α-Rha-(1→3)-2-*O*-Me-α-Rha-(1→3)-α-Rha-(1→3)-α-Rha-(1→3)-α-Rha-(1→2)-6-d-α-Tal. The Ku11-GPL was composed of 6 pieces of non-acylated sugars. We identified the two ORF*s* that are functionally responsible for the elongation of oligosaccharide, glycosyltransferase. Moreover, the intact GPLs in nature were acetylated at some positions of oligosaccharide, and recognized via toll-like receptor 2 (TLR2). In near future, we clarify the complete biosynthesis genes of Ku11-GPL, and control the structural and functional diversity of GPLs.

PT251 VapBC toxin-antitoxin modules of Sinorhizobium meliloti: actors of the nitrogen-fixing symbiosis

Camille Syska[1], Aurelie Kiers[1], Justine Lipuma[1], Isabelle Garcia[1], Laurence Dupont[1]

[1]*Institute Sophia Agrobiotech, Sophia Antipolis, France*

Background: The symbiotic interaction between *Sinorhizobium meliloti* and the model legume *Medicago truncatula* leads to the formation of new root organs, the nodules, where differentiated bacteria reduce the atmospheric nitrogen into ammonium.

Objectives: To better understand the intracellular lifestyle adaptation of bacteria during symbiosis, we examine the role of *S. meliloti* VapBC Toxin-Antitoxin (TA) systems. These TA systems are composed of a stable toxin (VapC) and a labile antitoxin (VapB) inactivating the toxin. In response to a signal, antitoxin degradation by bacterial proteases releases the toxin, acting as a post-transcriptional regulator due to its RNase site-specific activity.

Methods: Importance of VapBC modules was studied by phenotyping the interaction between *Medicago* and bacterial mutants deficient in the VapC toxin on their ability to nodulate, differenciate, fix nitrogen and persist in nodules. Lastly, identification of the consensus site of cleavage of representative VapC toxins by a RNA-seq method has been undertaken.

Results: Infection of *M. truncatula* with *vapC* mutants show that 4 mutants among 11 have an altered symbiotic phenotype: defect in root infection, early or delayed nodule senescence. This study demonstrates the overall importance of TA at all steps of symbiosis, making them essential actors in the plant-microbe interaction fitness. RNA-seq approach will contribute to identify the RNA targeted by specific VapCs, and to connect a defined symbiotic function to a specific VapBC module.

PT252 The distinct in vivo RNA global profiles of the Hfq, Hfq2 and ProQ from Burkholderia cenocepacia K56-2

Joana R Feliciano[1], Charlotte Michaux[2], Thorsten Bischler[3], Tiago Pita[1], Lars Barquist[4], Sílvia Andreia Sousa[1], António M.M. Seixas[1], Jörg Vogel[2;4], Jorge H. Leitão[1]

[1]iBB - Institute for Biotechnology and Biosciences, Instituto Superior Técnico, Universidade de Lisboa, Lisbon, Portugal
[2] Institute of Molecular Infection Biology, University of Würzburg, RNA Biology Group, Wuerzburg, Germany
[3]Core Unit Systems Medicine, University of Würzburg, Wuerzburg, Germany
[4]Helmholtz Institute for RNA-Based Infection Research (HIRI), Helmholtz-Centre for Infection Research (HZI), Würzburg, Germany

Background: The study of globally acting RNA-binding proteins (RBPs) has been intensified over the last years. Many of these proteins aid small non-coding regulatory RNA molecules (sRNAs) to bind directly to complementary sequences present in target mRNA molecules, and they are key components of post-transcriptional control networks. In bacteria, CsrA and Hfq are the two best studied RBPs. More recently, ProQ has also emerged as an important RBP involved in post-transcriptional control. The *Burkholderia cepacia* complex (Bcc) is a group of bacteria associated with a poor clinical prognosis in cystic fibrosis patients. These species have some of the largest bacterial genomes, and unlike most other bacteria, they encode two-distinct Hfq-like proteins and a ProQ-like chaperone.

Objectives: As the RBPs roles are poorly understood in the regulation of Bcc opportunistic pathogens, this work aims to unveil the specific cellular functions of the RNA chaperones Hfq, Hfq2 and ProQ of *B. cenocepacia*, seeking, to determine their molecular targets and their implications in the biology of these bacteria.

Methods: Using UV crosslinking-immunoprecipitation sequencing (CLIP-seq) methodology, Hfq and ProQ binding sites in the *B. cenocepacia* K56-2 transcriptome were globally profiled. In addition, the roles of each RBP on the *B. cenocepacia* physiology and virulence were assessed using mutant strains.

Results: While the *hfq* mutant strains display very pleiotropic phenotypes, less pronounced effects were observed in the absence of ProQ. The interactome of the three RBPs was unveilled and results suggest that distinct post-transcriptional networks are regulated by both Hfqs and ProQ.

PT253 Comparison of proteolytic processing of Saccharomyces cerevisiae cell wall proteins Scw4 and Scw10, and their evolutionary conservation among different yeast species

Antonija Grbavac[1], Mateja Lozančić[1], Renata Teparić[1], Vladimir Mrša[1]

[1]Faculty of Food Technology and Biotechnology, Laboratory for Biochemistry, Zagreb, Croatia

Background: Scw4p, one of noncovalently bound proteins in *Saccharomyces cerevisiae* cell wall, undergoes complex proteolytic processing by Kex2 and yapsins. It was suggested that this proteolytic processing can influence its cell wall incorporation, given that Scw4 can form covalent bond with β-glucan, and it's possible that processing has effect on Scw4 biological activity. Scw10 shares high level of similarity with Scw4, and also contains Kex2 processing site, but it remains unclear whether Scw10 is substrate for Kex2. Both of this proteins are considered to be glucanases, although this assumption hasn't been proven yet. Nevertheless, importance of this two proteins is indicated through extensive phenotypic changes caused by simultaneous deletion of both *SCW4* and *SCW10*. Their evolutionary preservation among different yeast species hasn't been investigated, however it's noted that genomes of some other yeast species contain *SCW4/SCW10* orthologs.

Objectives: Goals of this study were to explore Scw10 proteolytic processing and possible covalent binding to the wall, and to gather information and analyse evolutionary conservation of Scw4 and Scw10 among different yeast species.

Methods: Analysis were conducted using standard PCR and cloning techniques, western blot method and *in-silico* analysis of available proteomic data.

Results: Obtained results show that Scw10 undergoes processing by Kex2 and possibly by yapsins at the same site. This protein appears partially covalently bound to adjacent structures. Scw4 and Scw10 show high level of evolutionary preservation, given that majority of analysed yeast species contained at least one protein with high similarity to them.

PT254 Creation of heterologous expression system of anti-staphylococcal beta-lytic protease produced by Lysobacter capsici VKM-2533T

Irina Kudryakova[1], Alexey Afoshin[1], Darina Lyadova[2], Natalia Vasilyeva[1]

[1]PSCBR RAS G.K. Skryabin Institute of Biochemistry and Physiology of Microorganisms Russian Academy of Sciences, Pushchino, Russian Federation
[2]Mari State University, Yoshkar-Ola, Russian Federation

Background: Bacterial lytic enzymes demostrate a unique ability to hydrolyze peptydoglycan of bacteria that make these proteins highly perspective alternative to antibiotics as a new generation of efficient antimicrobial agents. We purified beta-lytic protease of *Lysobacter capsici* that turned out to be highly effecient against methicillin-resistant *Staphylococcus aureus* 55. The structure and functions of this protein have not been completely understood yet and thus the creation of the heterologous expression system of beta-lytic protease is extremely important and timely. However, this is not an easy task: the process is complicated by the «toxicity» against recombinant strains or aggregation as inclusion bodies, the problem common of all bacteriolytic enzymes.

Objectives: The creation of beta-lytic protease expression system.

Methods: PCR, cloning, expression of gene, refolding of protein, purification of recombinant protein, bioinformatics, SDS and agarose gel electrophoresis, bacteriolytic activity assay.

Results: We created two inducible expression systems on the basis of *Esherichia coli* cells (M15 and BL21(DE3)/pLysE) and plasmids (pQE30 and pET19(mod) containing the gene of beta-lytic protease without signal peptide, respectively. After gene expression, protease was accumulated by cells as an inclusion body. We selected the optimal conditions (buffers, temperature, and the time of protein activation) for refolding from the inclusion body to the active protein. In the result recombinant antistaphylococcal active beta-lytic protease was purification. The obtained expression system will be helpful in studies of the hydrolysis mechanism of bacterial living cells or peptidoglycan by beta-lytic protease at molecular and genetic levels necessary for the creation of new drugs.

PT255 Hexuronic acid metabolism: how UxuR and ExuR share their regulons?

Maria Tutukina[1;2], Inna Suvorova[1], Anna Kaznadzey[1], Uliana Shvyreva[2], Mikhail S Gelfand[1;3], Jeff Cole[4], Olga N Ozoline[2]

[1]*Institute for Information Transmission Problems RAS, Moscow, Russian Federation*
[2]*Institute of Cell Biophysics RAS, Pushchino, Russian Federation*
[3]*Skolkovo Institute of Science and Technology, Moscow, Russian Federation*
[4]*University of Birmingham, United Kingdom*

Background: The Ashwell pathway is important for bacterial motility and colonization of host organisms. UxuR and ExuR homologous proteins were predicted to control hexuronic acid metabolism, but little is known about their relative roles in gene regulation.

Objectives: The aim was to compare the UxuR and ExuR regulons on the genome-scale level and to reveal their interference with small RNAs.

Methods: ChiP-seq was used to map binding sites that were further confirmed with DNase I footprinting, RNA-seq, and qRT-PCR.

Results: A total of 365 UxuR targets were detected in the *E.coli* genome, of which eleven were absolutely prevalent and dependent on D-glucuronate, an intermediate of the Ashwell pathway. For ExuR, more than 300 targets were detected and this binding was much less dependent on the carbon source, suggesting that ExuR is a less specific global regulator. During growth with D-glucose, ExuR shares only 12% of its targets with UxuR, but on D-glucuronate 60% become common, possibly reflecting glucuronate-induced heterodimer formation. Despite proposed narrow specificity, UxuR regulon includes genes coding for FNR, Fis, ferric uptake systems and sRNAs.
UxuT is a 79 nt-long sRNA that initiates in the *uxuR* terminator and overlaps with short transcripts synthesized from within *uxuR* in antisense direction. Suprisingly, these transcripts were significantly overpresented in the fraction of extracellular RNAs secreted by *E.coli* during growth on M9/glucose. As such, the network of UxuR and ExuR regulons is much more complicated than expected and may involve novel class of regulatory RNAs.
The work was supported by RSF 18-14-00358

PT256 The role of Php1, a tyrosine phosphatase of Porphyromonas gingivalis, in community development with Streptococcus gordonii

Young-Jung Jung[1], Daniel Miller[2], Richard Lamont[2]

[1]Seoul National University School of Dentistry, Department of Oral Microbiology and Immunology, Seoul, Korea, Rep. of South
[2]University of Louisville School of Dentistry, Oral Immunology and Infectious Diseases, Louisville, United States

Background: Colonization of the oral cavity by *Porphyromonas gingivalis*, a keystone pathogen in the pathogenesis of periodontitis, can be facilitated by its interaction with an early colonizer *Streptococcus gordonii*, and protein tyrosine phosphoregulatory system in *P. gingivalis* can controls the early stage of *P. gingivalis-S. gordonii* heterotypic community development.

Objectives: The aim of this study was to investigate the role of Php1, a polymerase and histidinol polymerase domain-containing protein of *P. gingivalis*, in the community development with *S. gordonii*

Methods: The enzymatic activity of *P. gingivalis* Php1 was characterized using recombinant Php1 and substrates including p-nitrophenyl phosphate, phosphopeptides and recombinant proteins of *P. gingivalis* proteins. Production of extracellular polysaccharide and dual species (*P. gingivalis* ans *S. gordonii*) biofilm formation was examined using confocal microscopy.

Results: Recombinant Php1 (rPhp1) showed a tyrosine phosphatase activity in the presence of divalent metal ions, such as Mn^{2+}. rPhp1 dephosphorylated recombinant Ptk1 (rPtk1), a tyrosine kinase of *P. gingivalis*. The phosphatase activity was attenuated by mutations of conserved histidine residues, and mutation of a conserved arginine residue completely abrogated the Php1 activity. A *php1* mutant of *P. gingivalis* exhibited impaired community development with *S. gordonii* and extracellular polysaccharide production. *P. gingivalis* accretion into heterotypic community with *S. gordonii* and extracellular polysaccharide production were reinstated by complementation of *php1* mutant with the wild-type allele, but not by complementation with a phosphatase-inactive allele. Taken together, Php1, in particular its phosphatase activity, is required for *P. gingivalis-S. gordonii* community development.

PT257 Selection of Escherichia coli gentamycin resistant variant in biofilms exposed to polyhexamethylene biguanide (PHMB)

Arnaud Bridier[1], Cuzin Clémence[1], Moreau Marie-Hélène[1], Le Grandois Patricia[1], Soumet Christophe[1]

[1]*Anses, Fougères,*

Background: Antibiotic resistance is one of the most preoccupying issue facing modern medicine. Some biocides have demonstrated the potential of selecting resistance to antibiotics in bacteria, but data are still very scarce and it is important to better identify the molecules concerned and the underlying mechanisms.

Objectives: This study aimed to assess the potential of polyhexamethylene biguanide (PHMB), a widely used biocide in a variety of sectors, to select antibiotic resistance in *Escherichia coli* grown in biofilms.

Methods: Three *E. coli* strains isolated from pig industries and 1 collection strain were selected. Biofilms were grown on inox coupons and then daily exposed to sublethal concentrations of PHMB during 10 days. Antibiotic-resistant mutants were then selected and characterized phenotypically (resistance profiles against 14 antibiotics and 3 biocides, growth rate) and genotypically to identify mechanisms of resistance.

Results: Biocide exposition led to the selection of an *E. Coli* variant with a stable resistance to gentamycin (16-fold increase in Minimum Inhibitory Concentration (MIC) compared to Wild Type). This was also associated with a 40% decrease of growth rate of the variant. Susceptibility toward gentamycin was recovered in presence of the efflux pump inhibitor Phenylalanine-arginine β-naphthylamide (PAβN) in the variant. Moreover, *acrA*, a gene encoding an AcrAB-TolC multidrug efflux pump module was 5-fold overexpressed in variant. Together, these observations suggested a central role of AcrAB-TolC system in the cross-resistance observed. Sequencing and comparison of WT and variant whole genomes are currently in progress to go further in the deciphering of the underlying mechanism.

PT258 Generating the optimal messenger RNA vaccine platform for anthrax

Minhoon Lee[1], Na Young Kim[2], Young Kee Shin[3], Sungho Shin[4]

[1]Seoul National University, Graduate School of Convergence Science and Technology, Seoul, Korea, Rep. of South
[2]ABION Inc., R&D Institude, Seoul, Korea, Rep. of South
[3]Seoul National University, College of Pharmacy, Seoul, Korea, Rep. of South
[4]Seoul National University, Bio-MAX/N-Bio, Seoul, Korea, Rep. of South

Background: *Bacillus anthracis* is a gram-positive, spore-forming, rod-shaped, aerobic bacterium and the causative agent of the disease anthrax. Due to its highly pathogenic nature and long-lived spore forming capability, *B. anthracis* is considered as one of the most important biothreat agents. It is classified as category A bioweapon hazard by the Center for Disease Control.

Objectives: The current Food and Drug Administration licensed anthrax subunit vaccine has prolonged immunization schedule and adverse side effects. Therefore, the development of more potent anthrax vaccine platform is needed. Recently, mRNA vaccine technologies have emerged as promising alternatives to conventional vaccine approaches. In the present study, a mRNA vaccine vector was generated, which was optimized to induce high levels of anthrax fourth domain of protective antigen (PA-D4).

Methods: We designed a modified mRNA encoding the signal sequence from immunoglobulin M (IgM) and codon-optimized PA-D4 genes which has 70% similarity in terms of nucleic acid sequence. 1-methylpseudouridine-5'-triphosphate was used instead of UTP to generate modified nucleoside-containing mRNA. A modified mRNA also contained a type 1 cap and a 120 nucleotide-long poly(A) tail.

Results: Incubation of modified mRNA with 293T or NIH3T3 cells following lipoplex-mediated transfection resulted in efficient expression and secretion of 17 kDa of PA-D4, as detected by western blotting. Our results demonstrate that modified mRNA might be particularly useful to induce an immune response against anthrax infection.

PT259 Sigma B-dependent regulatory sRNA Rli47 represses isoleucine biosynthesis in Listeria monocytogenes

Catarina Moreira Marinho[1;2;3], Patrícia Teixeira dos Santos[4], Duarte Guerreiro[3], Birgitte H. Kallipolitis[4], Dmitry Ignatov[5], Jörgen Johansson[5], Pascal Piveteau[1;2], Conor O'Byrne[3]

[1]*Université de Bourgogne, Dijon, France*
[2]*Institut National de la Recherche Agronomique (INRA), UMR 1347 Agroécologie, Dijon, France*
[3]*National University of Ireland, Galway, Bacterial Stress Response Group, Galway, Ireland*
[4]*University of Southern Denmark, Department of Biochemistry and Molecular Biology, Odense, Denmark*
[5]*Umeå University, Department of Molecular Biology; Molecular Infection Medicine, Sweden (MIMS); Umeå Centre for Microbial Research, Umeå, Sweden*

Background: Small regulatory RNAs (sRNAs) can influence cellular processes by targeting mRNAs, leading to translation inhibition and mRNA degradation. Previous studies have shown that the σ^B-dependent sRNA Rli47 is induced in *L. monocytogenes* stationary phase cells, in the intestinal lumen and macrophages, but its function has remained elusive.

Objectives: This study aimed to comprehensively characterize Rli47 and determine its biological function in *L. monocytogenes*.

Methods: Besides *in silico* predictions, several molecular approaches such as DMS-MaPseq, northern blotting, EMSAs, RTqPCR, RNA-seq and phenotypic experiments were applied.

Results: Rli47 was shown to basepair through a single-stranded CU-rich loop with the ribosome binding site of *ilvA* mRNA, which encodes for threonine deaminase (TD), the first enzyme of the isoleucine biosynthetic pathway. Mutational analysis of the predicted binding regions on either Rli47 or *ilvA* mRNA abolished the basepairing. Rli47 was shown to reduce the expression of *ilvA*. A significantly shorter growth lag was found on Δ*rli47* and Δ*sigB* than the wild-type in isoleucine-depleted medium. Moreover, TD activity was higher in both mutant strains under this condition. Complementary transcriptomic analysis identified complex changes in gene expression in the Δ*rli47* mutant strain and highlighted a significant role for Rli47 in modulating amino acid metabolism. A potential role for Rli47 in the regulation of isoleucine biosynthesis emerged. Data point to a model where Rli47 is responsible for specifically repressing isoleucine biosynthesis even in conditions where it is absent, suggesting that isoleucine might act as a critical signal for *L. monocytogenes* at certain times during its life cycle.

PT260 L-arabinose induces D-galactose catabolism via the Leloir pathway in Aspergillus nidulans

Zoltán Németh[1], László Kulcsár[1], Michel Flipphi[1], Anita Orosz[1], Maria Victoria Aguilar-Pontes[2], Ronald P. de Vries[2], Levente Karaffa[1], Erzsébet Fekete[1]

[1]University of Debrecen, Department of Biochemical Engineering, Debrecen, Hungary
[2]Utrecht University, Fungal Physiology, Westerdijk Fungal Biodiversity Institute & Fungal Molecular Physiology, Utrecht, Netherlands

Background: L-Arabinose and D-galactose are the principal constituents of L-arabinogalactan, and also co-occur in other hemicelluloses and pectins.

Objectives: We hypothetized that similar to the induction of relevant glycoside hydrolases by monomers liberated from these plant heteropolymers, their respective catabolisms in saprophytic and phytopathogenic fungi may respond to the presence of the other sugar to promote synergistic use of the complex growth substrate.

Methods: To monitor the consumption of the two carbon sources in the wild-type and the corresponding D-galactose and L-arabinbose catabolic mutant strains we used submerged cultivation technics. We probed the transcription of the genes functionally involved in D-galactose and pentose catabolism, during batch cultivation of the wild type strain.

Results: We showed that these two sugars are indeed consumed simultaneously by Aspergillus nidulans, while L-arabinose is utilised faster in the presence than in the absence of D-galactose. Furthermore, the first two genes of the Leloir pathway for D-galactose catabolism – encoding D-galactose 1-epimerase and galactokinase – are induced more rapidly by L-arabinose than by D-galactose eventhough deletion mutants thereof grow as well as a wild type strain on the pentose. D-galactose 1-epimerase is hyperinduced by L-arabinose, D-xylose and L-arabitol. The results suggest that in A. nidulans, L-arabinose and D-xylose – both requiring NADPH for their catabolism – actively promote the enzyme infrastructure necessary to convert beta-D-galactopyranose via the Leloir pathway with its alphaanomer specific enzymes, into beta-D-glucose-6-phosphate even in the absence of D-galactose.

This work was supported by the EU and co-financed by the European Regional Development Fund [GINOP-2.3.2-15-2016-00008].

PT261 Microbiology based diagnosis of human brucellosis in herzegovina-neretva canton in bosnia-herzegovina

Maja Ostojic[1], Jurica Arapovic[1], Silvio Spicic[2], Sanja Duvnjak[2], Andrea Boskovic[3], Zeljko Cvetnic[2]

[1]*University Clinical Hospital Mostar, Bosnia and Herzegovina*
[2]*Croatian Veterinay Institute, Zagreb, Croatia*
[3]*Faculty of pharmacy and biochemistry, Zagreb, Croatia*

Background: Human brucellosis is still the most common bacterial zoonosis worldwide. The infection caused by the bacterial genus *Brucella,* is transmitted to humans from infected domestic animals, through ingestion of contaminated animal products as well as through handling *Brucella* isolates in laboratories.

Objectives: It is very important to establish the diagnosis of brucellosis. Blood culturing of *Brucella* is time-consuming and considered to be unreliable. Thus, diagnosis is often based on indirect serological tests and molecular typing.

Methods: This study included 16 patients that were admitted to and treated at the Department of Infectious Diseases of the University Clinical Hospital Mostar, Bosnia-Herzegovina, between 2015 and 2017. In this study, we investigated only microbiological parameters. The Rose Bengal test (RBT) positive sera were serologically confirmed by complement fixation test (CFT). We also analyzed blood cultures, and isolates were serotyped by molecular typing methods: Bruce-ladder multiplex polymerase chain reaction (PCR) and multiple locus variable number of tandem repeat analysis of 16 loci (MLVA-16) assay.

Results: RBT was used as a screening test for *Brucella spp.,* and all 16 patients were confirmed as a positive. The serum samples were found as a positive in 15/16 (93.7%) when we employed the CFT. The blood cultures were microbiologically positive for bacterial growth in 13/16 (81.3%) patients, and additionally serotyped as biovar 3. Using MLVA-16 assay, 11 isolates were genotyped. We observed complete genotype matches among 8/11 B.melitensis isolates, while 3/11 isolates differed in Bruce04 locus.

PT262 Immunobiotic Lactobacillus jenseni TL2937 alleviates dextran sodium sulfate-induced colitis in mice

Aminul Islam[1;2;3], Valeria Garcia-Castillo[4;5], Nana Sato[1;2], Leonardo Albarracin[1;4;6], Haruki Kitazawa[1;2], Julio Villena[1;4]

[1]*Food and Feed Immunology Group, Graduate School of Agricultural Science, Tohoku University, Sendai, Japan*
[2]*International Education and Research Centre for Food and Agricultural Immunology (CFAI), Graduate School of Agricultural Science, Tohoku University, Sendai, Japan*
[3]*Department of Medicine, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh*
[4]*Laboratory of Immunobiotechnology, Reference Centre for Lactobacilli, (CERELA-CONICET), Tucuman, Argentina*
[5]*Laboratory of Bacterial Pathogenicity, Faculty of Biological Sciences, University of Concepcion, Concepcion, Chile*
[6]*Laboratory of Computing Science. Faculty of Exact Sciences and Technology. Tucuman University, Tucuman, Argentina*

Background: immunobiotics have emerged as promising interventions to alleviate inflammatory damage in inflammatory bowel disease (IBD). However, the beneficial properties of immunobiotics are strain dependent and therefore, each strain has to be evaluated in order to demonstrate its potential application in IBD. *Lactobacillus jensenii* TL2937 attenuates gut acute inflammatory response triggered by Toll-like receptor 4 activation. However, its effect on colitis has not been evaluated before.

Objectives: the aim of this work was to study whether the TL2937 strain was able to protect against the development of colitis in a dextran sodium sulfate (DSS)-induced mouse model.

Methods: *L. jensenii* TL2937 was administered to five-week-old female BALB/c mice before (4 days) the induction of colitis, at a dose of 10^8 cells/mouse/day. Untreated mice were used as controls. Colitis was established by the administration of 2.5% (w/v) DSS in drinking water for 7 days. Mice were examined daily during 18 days for body weight, general appearance, stool consistency, and the presence of fecal blood. In addition, myeloperoxidase activity and cytokine production were evaluated in colon tissue samples.

Results: Mice fed with *L. jensenii* TL2937 had lower disease activity index and alterations of colon length when compared to control mice. In addition, reduced myeloperoxidase activity, lower production of pro-inflammatory (TNF, IL-1, IL-8, MCP-1, IL-15 and IL-17) and higher levels of immunoregulatory (IL-10 and IL-27) cytokines were found in TL2937-treated mice. These findings indicate that *L. jensenii* TL2937 is able to alleviate DSS-induced colitis and suggest a novel application for this probiotic strain.

PT263 From waste to platform chemicals: a yeast GWAS pipeline

Joseph Shepherd[1], Tom Clarke [1], Jo Dicks [1], Matthew Hutchings[1], Jonathan Todd[1]

[1]*University of East Anglia, Quadram Institute Building and the John Innes Centre, United Kingdom*

Background: With falling oil reserves and global warming, chemicals once synthesised from crude oil must be obtained through alternative technologies. Consequently, many teams have attempted to obtain biofuels and other chemicals through microorganisms' fermentative processes, using glucose or other carbon sources as feed stocks. However, using food crops as feed stocks diverts land usage to fuel/chemical production, causing an increase in food prices. Therefore, a method to utilise the carbon locked in parts of crops that are not consumed, such as lignocellulosic biomass, would be essential.

Objectives: To identify strains in the National Collection of Yeast Cultures (~3000, of which 1000 are genotyped) that generate increased amounts of desired platform chemicals (e.g. Succinate) and identify strains that are also resistant to inhibitors generated during pre-treatment of lignocellulose. The strains can be ranked to those both resistant to the pre-treatments, with high production of specific chemicals. Once the specific biochemical pathways have been identified in a genome-wide manner, they can be elucidated further.

Methods: Separate experiments for optical density (OD) for resistance to furfuraldehyde (common pre-treatment byproduct) were conducted. Next, triplicate NMR quantification of metabolic products of each strain was performed in minimal media in fermentative conditions.
Strains were then run through a genome-wide association study (GWAS) pipeline, using a Manhattan plot to visualise corrolation of individual SNPs to desired phenotype.

Results: Some promising strains and SNPs have been identified, with ideas for going forwards.

PT264 Metabolic regulation of antibiotic resistance in Pseudomonas aeruginosa

Elisabeth Sonnleitner[1], Armin Resch[1], Udo Bläsi[1]

[1]*University of Vienna, Dept. of Microbiology, Immunobiology and Genetics, Vienna, Austria*

Background: In the opportunistic human pathogen *P. aeruginosa,* several uptake functions for amino-acids and carbon sources serve simultaneously as entry ports for antibiotics. The respective genes are often controlled by carbon catabolite repression (CCR). We have recently shown that Hfq and the catabolite repression control protein (Crc) translationally repress target mRNAs during CCR. This function is counteracted by the regulatory RNA CrcZ, which acts as a decoy for Hfq.

Objectives: *P. aeruginosa* is responsible for approximately 10% of hospital-acquired infections worldwide. It is notorious for its high level resistance towards many antibiotics and multi drug resistance of clinical isolates is steadily increasing. Understanding the molecular mechanism underlying drug resistance is crucial for the development of novel antimicrobial agents or alternative strategies such as enhanced sensitization to antibiotics in use.

Methods: To gain better insights into the role of Hfq in antibiotic resistance in *P. aeruginosa*, we determined the minimal inhibitory concentration (MIC) of PA14 and its isogenic *hfq* deletion mutant. RNAseq analysis and further molecular investigations revealed that Hfq/Crc controls several functions associated with antibiotic resistance.

Results: Here, we report an increased susceptibility of the *P. aeruginosa hfq* deletion strain to different classes of antibiotics. Furthermore, we will discuss how these insights on Hfq/Crc/CrcZ mediated regulation can be harnessed to increase Hfq/Crc-dependent antibiotic susceptibility of *P. aeruginosa*.

PT265 Amyloid-forming properties of Cupin-1 domain of P. sativum seed storage protein produced in bacteria and yeast

Mariia Belousova[1], Mikhail Belousov[1;2], Kirill Antonets[1;2], Oksana Shtark[3], Anastasiia Kosolapova[1;2], Anton Nizhnikov[1;2]

[1]All Russia Research Institute for Agricultural Microbiology, Laboratory for Proteomics of Supra Organismal Systems, Saint-Petersburg, Russian Federation
[2]Saint Petersburg State University, Department of Genetics and Biotechnology, Saint-Petersburg, Russian Federation
[3]All Russia Research Institute for Agricultural Microbiology, Saint-Petersburg, Russian Federation

Background: Proteins possessing amyloid forming properties are mostly associated with fatal human and animal diseases. However, the amyloids with vitally important roles were found recently in archaea, bacteria, fungi and animals. Higher plants are the least studied group of organisms in the field of amyloid biology.

Objectives: The goal of investigation was to prove amyloid-forming properties of P. sativum Cupin-1 domain produced in bacterial and yeast systems.

Methods: Prediction of potentially amyloidogenic regions was performed and Waltz and SARP bionformatic algorithms. Analysis of amyloid properties of Cupin 1 domain was performed in Curli-Dependent Amyloid Generator (C-DAG) system, which allows to export a protein of interest to the surface of E. coli cells.

Results: Proteomes of 75 plant species were analyzed using Waltz and SARP algorithms. Based on the data obtained, glutamine- and asparagine-rich seed storage globulins of higher plants were chosen for further evaluation. Evolutionary conservative Cupin-1 domain of Pisum sativum 7S globulin was produced in bacteria and its ability to aggregate was shown. E. coli cells exporting Cupin-1 exhibit typical for amyloids apple-green birefringence in polarized light upon Congo Red dye binding. Fibrillary morphology of the Cupin-1 aggregates was shown using transmission electron microscopy. Furthermore, Cupin-1 fused with YFP aggregates in yeast cells. Talking together, we conclude that Cupin-1 domain from Pisum sativum 7S globulin has amyloid-forming properties. This work was supported by the Russian Science Foundation (Grant No 17-16-01100).

PT266 Mechanisms of fosfomycin collateral susceptibility of Pseudomonas aeruginosa antibiotic resistant mutants

Pablo Laborda-Martínez[1], Sara Hernando-Amado[1], Jose Luis Martinez[1]

[1]*Centro Nacional de Biotecnología - CSIC, Madrid, Spain*

Background: *Pseudomonas aeruginosa* is a nosocomial pathogen that presents low antibiotic susceptibility. In addition, it acquires increased antibiotic resistance, being mutation-driven resistance particularly relevant in the case of chronic infections. Using experimental evolution approaches, we detected that tobramycin, tigecycline and ceftazidime *P. aeruginosa* resistant mutants presented collateral susceptibility to fosfomycin, but the reasons behind such hypersusceptibility were unknown.

Objectives: The purpose of this study was to determine the underlying mechanism of the collateral susceptibility to fosfomycin of *P. aeruginosa* resistant mutants selected upon tobramycin, tigecycline or ceftazidime challenge.

Methods: Three individual clones, from each of the fosfomycin hypersusceptible populations, were isolated and Sanger sequenced. In addition, a RNA-seq transcriptional analysis was performed to infer transcriptional alterations that may be involved in this phenotype.

Results: No mutations in the known fosfomycin resistance determinants were noticed. However, the genes encoding the peptidoglycan recycling pathway, which contribute to the intrinsic fosfomycin resistance, and *fosA*, encoding an intracellular fosfomycin inactivating enzyme, were expressed at lower level in the mutants than in the parental wild-type strain. These results suggest that a reduced expression of intrinsic fosfomycin resistance genes could be in the basis of the collateral susceptibility phenotype observed. Further research is needed to ascertain the potential use of fosfomycin, together with the antibiotics above mentioned, in cycling treatment of *P. aeruginosa* infections.

PT267 Acquired fosfomycin resistance in Stenotrophomonas maltophilia by mutations in central carbon metabolism enzymes

Teresa Gil[1], Jose Luis Martinez[1], Fernando Corona[1], Alejandra Bernardini[1]

[1]*Centro Nacional de Biotecnología, CSIC, MICROBIAL BIOTECHNOLOGY, Madrid, Spain*

Background: *Stenotrophomonas maltophilia* is an opportunistic pathogen characterized by a reduced susceptibility to currently used antibiotics. Consequently, the treatment of infections caused by *S. maltophilia* is complicated. Although fosfomycin is used for the treatment of such infections, the fosfomycin resistance mechanisms in this bacterium are unknown.

Objectives: The main objective of this work is the study of fosfomycin resistance mechanisms in mutants of *S. maltophilia* D457 isolated in the laboratory in presence of this antibiotic.

Methods: Fosfomycin resistant *S. maltophilia* D457 mutants were isolated in MH agar plates in presence of fosfomycin. The genome of a selected set of mutants was sequenced using Illumina technologies. Complementation of the mutants was carried out by mating. Intracellular accumulation of fosfomycin was measured using a bioassay. Functional consequences of these mutations were analysed by measuring in each mutant the activity of the enzymes encoded by the mutated genes. Transcriptomic analyses were performed by means of RNAseq.

Results: None of the mutants presented a decreased amount of intracellular fosfomycin, indicating that the mechanism of resistance is not the consequence of alterations in the transport or inactivation of the antibiotic. All mutants present mutations in genes encoding different enzymes of the Embden-Meyerhof-Parnas (EMP) pathway. These mutations correlate with the inactivation of corresponding enzymes as measured biochemically. Differing to other organisms in which the cause of fosfomycin resistance is a reduced intracellular concentration of the antibiotic, metabolic remodelling due to mutations in EMP enzymes is in the basis of *S. maltophilia* fosfomycin resistance.

PT268 Reversible lysine acylation modulates the activity of acetyl-CoA synthetase (Acs) in Staphylococcus aureus

Rachel Burckhardt[1], Jorge Escalante-Semerena[1]

[1]*University of Georgia, Microbiology, Athens, United States*

Background: Lysine acylation is a posttranslational modification used by all domains of life to modulate cellular processes. The paradigm for the role of reversible lysine acylation in metabolism is the acetyl-coenzyme A synthetase (Acs) enzyme. In prokaryotic and eukaryotic cells alike, Acs activity is down regulated by acylation of an active site lysine by an acetyltransferase enzyme. Deacetylase enzymes remove the acyl group to restore activity. Here we show modulation of Acs activity by reversible lysine acylation in *Staphylococcus aureus*.

Objectives: To characterize the role of acylation of the acetyl-CoA synthetase enzyme in *Staphylococcus aureus*.

Methods: Phosphor imaging was used to monitor the transfer of radiolabelled acetyl moieties onto *Sa*Acs by *Sa*AcuA. Removal of the label by the *Sa*CobB sirtuin deacylase was used to determine the reversibility of the process. *Sa*Acs activity was monitored using a continuous spectrophotometric assay. *Sa*Acs was purified from *S. aureus acuA*$^+$ and *acuA* strains and the level of Acs activity was quantified. *Sa*Acs and *Sa*CobB functionality was assessed *in vivo* using well-characterized strains of *Salmonella enterica*.

Results: We report experimental evidence to validate the function of *Sa*Acs as a *bona fide* acetyl-CoA synthetase enzyme. *Sa*Acs can be inactivated by acylation by the acetyltransferase *Sa*AcuA, which can use a variety of acyl-CoAs, including succinly-CoA, as substrates. *Sa*AcuA is the first example of a bacterial *N*-acetyltransferase that can succinylate proteins. *Sa*CobB removed the acetyl group, thus restoring enzyme activity. Together, these proteins form a reversible lysine acylation system that regulates Acs activity in *Staphylococcus aureus.*

PT269 Characterization of formate dehydrogenase of Shewanella amazonensis

Kabiru Usman[1], Tom Clarke[1], Richard Bowater [1]

[1]*University of East Anglia, Department of Biological Sciences, United Kingdom*

Background: Members of *Shewanellacea* have the ability to utilise formate as an electron donor for ATP generation under anaerboic respiration. A preliminary study conducted on different strains of *shewanella* species shows that the *S. amazonensis* formate dehydrogenase has a low catalytic activity of formate oxidation, and a high carbon dioxide reductase activity using methyl viologen as an electron acceptor or donor.

Objectives: The aim of this research was to characterize formate dehydrogenase of *S. amazonensis* and to investigate its possible use as a biocatalyst for the reduction of CO_2 to formate. The growth phenotypes of *S. amazonensis* was compared to *S. oneidensis* on formate under aerobic and anaerobic conditions, and the catalytic properties of the formate dehydrogenase from *S. amazonensis* were investigated further to better understand the preliminary data.

Methods: *S.amazonensis* and *S.oneidensis* cells were grown aerobically in LB media supplemented with formate. Minimal media supplemented with formate and fumarate was used to study the ability of the strains to utilize formate and fumarate. Methyl viologen was used to study formate oxidation and carbon dioxide reduction using LB and minimal media under 3 different conditions.

Results: Results show that excess formate cannot support growth of *S.amazonensis* and *S.oneidensis* aerobically in LB media. The growth decreases as the concentration of formate increases. Anaerobic growth on Minimal media supplemented with formate and fumarate suggested that the *S. amazonensis* growth rate was lower than the growth rate of *S. oneidensis*. Kinetic assays suggest a lower V_{max} was responsible for the observed kinetic differences.

PT270 Apoptosis-like cell death of quorum sensing-defective mutants of Burkholderia glumae

Yewon Nam[1], Yongsung Kang[1], Ingyu Hwang[1]

[1]Seoul National University, Department of Agricultural Biotechnology, seoul, Korea, Rep. of South

Background: The rice pathogen *Burkholderia glumae* utilizes amino acids as nutrient sources for the growth and produces lots of ammonia as results of deamination of amino acids during growth. Thus, they produce oxlate as a pulbic good in a quorum sensing (QS)-dependent manner to neutralize alkaline environmental pH. The QS-defective mutants of *B. glumae* experience alkaline stress due to lack of oxalate and confront cell death at stationary phase.

Objectives: We aimed to determine whether cell death of QS mutants of *B*. *glumae* due to alkaline stress is genetically coordinated processes as if apoptosis-like cell death in other bacteria.

Methods: Cells of *B. glumae* were exposed to alkaline stress followed by monitoring cellular changes by using cell biological methods such as DAPI, Live/Dead, $DiBAC_4$ and SYTO RNA select fluorescent cell staining during cell growth. Hallmarks of apoptosis-like cell death such as membrane depolarization and levels of reactive oxygen species (ROS) were measured.

Results: Typical phenomena of apoptosis-like cell death such as cell membrane depolarization, DNA fragmentation, and increase of ROS were observed in QS mutants and wild type of *B. glumae* under alkaline stress. Cellular materials such as DNA, RNA, and proteins were degraded in cells exposed to alkaline stress conditions. These results show genetically-coordinated processes similar to bacterial apoptosis-like cell death of *B*. *glumae* in response to alkaline stress. We demonstrate the first case of apoptosis-like cell death triggered by alkaline stress in bacteria.

PT271 Genome diversity and social activities of Burkholderia glumae

Minhee Kang[1], Eunhye Goo[1], Jae Yun Lim[2], Jinwoo Kim[3], Jeong-Ah Seo[2], Ingyu Hwang[1]

[1]Seoul National University, Department of Agricultural Biotechnology, Seoul, Korea, Rep. of South
[2]Soongsil University, School of Systems Biomedical Science, Seoul, Korea, Rep. of South
[3]Gyeongsang National University, Division of Applied Life Science and Institute of Agricultural and Life Science, Jinju, Korea, Rep. of South

Background: The rice pathogen *Burkholderia glumae* has two chromosomes and four plasmids with a total size of 7.2 Mb. Important pathogenicity genes such as Hrp type III secretion and toxoflavin biosynthesis genes are in the chromosome 2. The bacterium possesses a single LuxI-R type quorum sensing (QS) system and produces oxalate as a public good in a QS-dependent manner to counteract ammonia-mediated alkaline environmental pH.

Objectives: We aimed to determine whether genome diversity of isolates originated from different ecological niches influences social activities of *B. glumae*.

Methods: Genome information of a total 58 isolates of *B. glumae* obtained from diseased rice panicles, broken rice, and solanaceae crops was determined. QS signals and toxoflavin were analyzed on TLC plates, and levels of oxalate production were measured by using oxalate assay kits.

Results: Significant differences in genome structures such as inversion, rearrangement, and mergence of chromosomes were found among isolates. Most isolates obtained from diseased broken rice did not produce toxoflavin and were avirulent. In toxoflavin-defective isolates, we found a large DNA fragment insertion in the promoter region of toxoflavin biosynthetic gene cluster. Most of toxoflavin-defective isolates were resistant to kanamycin and spectinomycin and more competent than than a prototype isolate BGR1. Those isolates produced less QS signals and oxalate than BGR1, which caused alkaline environment due to massive generation of ammonia upon growth in Luria-Bertani medium. These results indicate that genome plasticity of *B. glumae* affects its social activities.

PT272 Characterization of a T6SS effector protein of Serratia marcescens Db10

Ramses Gallegos Monterrosa[1], Francesca Cianfanelli[2], Sarah Coulthurst[3]

[1]School of Life Sciences - University of Dundee, Division of Molecular Microbiology, Dundee, United Kingdom
[2]Biozentrum - Center for Molecular Life Sciences, University of Basel, Switzerland
[3]School of Life Sciences - University of Dundee, Division of Molecular Microbiology, Dundee, United Kingdom

Background: The Type 6 secretion system (T6SS) is a versatile protein delivery mechanism used by many Gram-negative bacteria as a weapon in inter-microbial and host-microbe interactions. The T6SS is recognized as an important competition factor in bacteria that significantly affects their environmental fitness. *Serratia marcescens* is a Gram-negative oportunistic pathogen that can be found in diverse niches and displays a functional T6SS. *S. marcescens* possesses several toxic effector proteins that can be delivered via its T6SS, and corresponding immunity factors that protect it from self-intoxication. Slp (Secreted lipase-like protein) was recently reported as a T6SS effector in *S. marcescens*, along with two putative immunity factors.

Objectives: To characterize the biological and mechanistic role of Slp, a novel T6SS-dependent effector protein of *S. marcescens*.

Methods: We use a combination of molecular biology and microbiology techniques to study the biological role of Slp, its mechanism of action, and the method of self-protection used by *S. marcescens*: experimental bacterial competition against other *S. marcescens* strains and diverse bacterial species, lipidomics analyses to study the putative enzymatic properties of Slp, and a biochemical approach to investigate the interactions between Slp and its putative immunity factors.

Results: The biological characterization of Slp showed a differential sensitivity among Gammaproteobacteria to this effector, and established the protection conferred upon *S. marcescens* by one of the reported immunity factors. Our results reveal the role that Slp plays within the context of T6SS-mediated inter-bacterial competition in *S. marcescens.*

PT273 Understanding Escherichia coli formte channels working direction during fermentation of mixture of glucose, glycerol and formate at pH 7.5

Karen Trchounian[1], Bella Hakobyan[1], Lusine Karapetyan[1], Armen Trchounian[1]

[1]*Yerevan State University, Biochemistry, Microbiology and Biotechnology, Yerevan, Armenia*

Background: *Escherichia coli* produces molecular hydrogen (H_2) via oxidation of formate catalyzed by formate hydrogen lyase complex. It is known that formate is transported through FocA and FocB channels. During fermentation FocA has an important role in regulating intracellular formate level. Role of FocB is not clear.

Objectives: Main goal of the work was to understand the physiological role and working direction of formate channels during mixed carbon fermentation at pH 7.5.

Methods: Electrochemical methods of H_2 production has been used, particularly Pt- and Ti-Si redox electrodes to measure and detect H_2 generation by bacteria.

Results: It is shown that at pH 7.5 when cells were grown in the presence of glucose and glycerol mixture and in the assays glucose was added in Δ*focB* and Δ*focA focB* H_2 production increased, compared to wild type suggesting that channels are working in exporting direction. In glycerol assays H_2 production in Δ*focA* increased ~3-fold and in Δ*focB* decreased ~1.7-fold. When cells were grown in the presence of glucose, glycerol and formate mixture and in the assays glucose was added in *focA* and *focB* H_2 production increased ~3-fold and ~2.5-fold, respectively, compared to wild type. In formate assays in *focA* and *focA focB* H_2 production was increased ~1.3- and ~1.7-fold, respectively. Moreover, in *focB* mutant it was decreased ~2-fold.

Taken together the data suggest that the presence of external formate changes the working direction of formate channels which might be due to maintaining, H_2 and proton cycling and thus cytoplasmatic pH.

PT274 Genetic heterogeneity among Xanthomonas campestris pv. campestris isolates originated from oilseed rape determined with different rep-PCR techniques

Aleksandra Jelušić[1], Tatjana Popović[2], Petar Mitrović[3], Ivica Dimkić[4], Slaviša Stanković[4], Sanja Marković[5], Tanja Berić[4]

[1]*Institute for multidisciplinary research, Belgrade, Serbia*
[2]*Institute for Plant Protection and Environment, Belgrade, Serbia*
[3]*Institute of Field and Vegetable Crops, Novi Sad, Serbia*
[4]*Faculty of Biology, University of Belgrade, Belgrade, Serbia*
[5]*Deka inžinjering, Belgrade, Serbia*

Background: Oilseed rape (*Brassica napus*) is an oil-producing crop, significant for vegetable oil and biodiesel production. The phytopathogenic bacterium *Xanthomonas campestris* pv. *campestris* (*Xcc*) causing bacterial leaf blight was recorded for a first time in 2010 on self-growing winter oilseed rape in Serbia. Ever since, it continued appearing on cultivated crops too.

Objectives: The aim of this research was to reveal genetic heterogeneity among 25 *Xcc* isolates, originated from different cultivars of winter oilseed rape obtained in nine-year period (2010-2018) from different localities in Vojvodina region (Serbia) using repetitive sequence-based PCR (rep-PCR) fingerprinting.

Methods: Genomic DNA was extracted using CTAB method. Specific primer pair XCF/XCR, amplifying a DNA fragment of *hrpF* gene from *X. campestris* was used for rapid preliminary identification of all tested isolates. Rep-PCR using BOX, ERIC and REP-PCR primers was used to detect presence of genetic heterogeneity among *Xcc* isolates.

Results: All 25 tested isolates were preliminary identified as *Xcc* based on a positive PCR reaction after amplification of 525 bp region using XCF/XCR primers. BOX and ERIC-PCR generated seventeen different DNA fingerprinting profiles, unlike REP-PCR where only nine different profiles were detected. These results show presence of genetic polymorphism among *Xcc* isolates from winter oilseed rape, which could indicate either their affiliation to some of the identified *Xcc* races, or formation of a new *Xcc* ecotype.

PT275 The small non-coding RNA RyhB homolog participates in the physiology and virulence of Yersinia ruckeri

Lillian Gabriela Acuña Olivares[1], María José Barros[1], Paula Nuñez[1], Fernanda Montt[1], Juan Fuentes[1], Iván Luis Calderón Lizana[1]

[1]*Universidad Andres Bello, Laboratorio de Genética y Patogénesis Bacteriana, Facultad Ciencias de la Vida, Santiago, Chile*

Background: *Yersinia ruckeri* is a Gram-negative bacterium responsible for enteric redmouth disease, a serious septicemic disease that affects salmonids.

In *Y. ruckeri* pathogenesis the low availability of iron is critical and therefore bacterium must to activate mechanisms to optimize its use. Among these mechanisms, small noncoding RNAs (sRNA) are crucial regulators of many physiological processes. RyhB is a sRNA conserved in many Enterobacteriae whose expression is induced under iron starvation. We sequenced the genome of a *Y. ruckeri* strain isolated from a salmon farm in Chile and we identified the presence a homolog of RyhB (RyhByr1).

Objectives: Analyze the participation of the homolog RyhB in the physiology and virulence of *Yersinia ruckeri*

Methods: We constructed a strain lacking RyhByr1 by homologous recombination and the replication status, motility and ATP levels several parameters was monitored in iron-limited cultures. The expression of RyhByr1 and mRNA targets (predicted *in silico*) was analyzed by qRT-PCR. The participation of RyhByr1 in the infective process was analyzed by *in vitro* infection assays.

Results: The RyhByr1 mutant strain exhibited a hypermotile phenotype, increased rates of growth and replication and augmented intracellular ATP levels in iron-limited cultures. Also showed an increased proliferation in intracellular cultures of salmonid macrophages. RyhByr1 expression under iron-limited cultures was confirmed and the putative targets in the mutant strain showed increased expression in the case of metabolic (*sdhC, cysE, acnB, atpC*) and cell division genes (*ftsX, ftxL, mraZ*). These results indicate that RyhByr1 participates in the modulation of physiology and virulence of *Y. ruckeri.*

PT276 Insights into colibactin regulation: Escherichia coli`s elusive genotoxin

Alexander Wallenstein[1], Rolf Müller[2], Ulrich Dobrindt[1]

[1]Institute of Hygiene, Westfälische Wilhelms-University and University Hospital Münster, Microbial Genome Plasticity, Münster, Germany
[2]Helmholtz-Institute for Pharmaceutical Research Saarland, Department of Microbial Natural Products, Saarbrücken , Germany

Background: A variety of Escherichia coli, including commensals and pathogenic isolates, contain a genomic island, encoding the biosynthetic machinery for the non-ribosomal peptide/polyketide colibactin. While the biological role of colibactin remains under discussion, it could be linked to probiotic as well as genotoxic effects. Although purification of full length colibactin remains challenging, its mode of action was already described: its genotoxic effect is caused by inducing DNA double strand breaks. We address the biological role of colibactin by studying regulation of colibactin production revolving around the essential regulator ClbR.

Objectives: We wanted to gain insights into the regulatory network and elements affecting the expression of the colibactin determinant. Especially, we investigated the impact of the essential transcriptional activator ClbR and the key thioesterase ClbQ on the transcription of the colibactin island and on resulting polyketide expression.

Methods: Aiming to understand regulation of colibactin expression and its interconnection with cellular regulatory networks, we utilized RNA-seq and mass spectometry to analyse transcription of the colibactin island as well as expression levels of cellular intermediates of colibactin synthesis and of the proteins encoded by the colibactin island. In addition we compared resulting colibactin levels indirectly by monitoring cythopathic effect via γH2AX Western blots.

Results: Our work identified ClbR as specific transcriptional autoactivator, which increases colibactin production, and also identified factors affecting ClbR regulation. Additionally, we describe a correlation between colibactin synthesis and ClbQ expression further increasing our understanding of different levels involved in the complex regulation of colibactin expression.

PT277 Next-generation sequencing-based transcriptome analysis of Streptococcus zooepidemicus during hyaluronic acid fermentation

Matouš Čihák[1;2], Jana Jílková[1], Zbyněk Černý[1], Sofia Chatzigeorgiou[1;2], Kristýna Šinovská[1], Jan Bobek[2;3], Vladimír Velebný[1]

[1]*Contipro Inc., Dolní Dobrouč, Czech Republic*
[2]*Charles University, First Faculty of Medicine, Institute of Immunology and Microbiology, Prague, Czech Republic*
[3]*J. E. Purkinje University, Chemistry Department, Faculty of Science, Ústí nad Labem, Czech Republic*

Background: Hyaluronic acid is a high molecular weight biopolysaccharide that occurs naturally in the human body. It acts as a major component of the extracellular matrix and hydrating substance. The predominant characteristic of hyaluronic acid is its significant water-binding capacity which results in biological properties and predestines the molecule to numerous applications in pharmaceutical and other industries.

Objectives: *Streptococcus equi* subsp. *zooepidemicus* is currently the most exploited bacterium in the biotechnological production of hyaluronic acid. Although this producer is a frequent object of scientific research, the complex transcriptome of *S. zooepidemicus* during fermentation has not been extensively studied yet. Because such a research may clarify processes taking place inside a bacterial cell, we focused on the next-generation sequencing-based transcriptome analysis in *S. zooepidemicus*.

Methods: Using high-throughput sequencing we identified the genes being expressed after 16th hour of the cultivation in complex medium.

Results: From the acquired results we conclude that the expressed genes may be assigned to the following biological functions: stress response, energy production and conversion, amino acid transport and metabolism, regulation of transcription and translation, cell signaling and, lastly, virulence factors. Data analysis allowed us gain knowledge about the physiological state of *S. zooepidemicus* culture after 16 hours of cultivation. Even though the concentration of hyaluronic acid reaches its apex at this timepoint, the hyaluronic acid biosynthetic genes are rather lowly expressed. In summary, we were able to describe gene expression pattern during the hyaluronic acid fermentation by *S. zooepidemicus* using high-throughput sequencing for the first time.

PT278 Identification of novel amyloid-forming proteins RopA and RopB in the root nodule bacterium Rhizobium leguminosarum

Anastasiia Kosolapova[1;2], Kirill Antonets[1;2], Mikhail Belousov[1;2], Maria Belousova[1], Anna Lykholay[2], Oksana Shtark[1], Ekaterina Vasileva[1;2], Kirill Volkov[2], Anton Nizhnikov[1;2]

[1]All-Russia Research Institute for Agricultural Microbiology, St. Petersburg, Pushkin, Russian Federation
[2]St. Petersburg State University, St. Petersburg, Russian Federation

Background: Amyloids represent non-soluble protein aggregates formed by unbranching fibrils characterized by cross-β structure. The amyloid formation is associated with development of more than 40 fatal diseases including Alzheimer's disease. Nevertheless, investigations of last decades demonstrated that amyloids can perform various physiological functions. The majority of functional amyloids of prokaryotes were identified within *Gammaproteobacteria* species including *Escherichia coli*. However, no functional amyloids have been identified within *Alphaproteobacteria* species yet.

Objectives: We aimed to investigate novel functional amyloids of *Rhizobium leguminosarum* (class *Alphaproteobacteria*) – agriculturally important symbiont of legumes.

Methods: To identify potential amyloidogenic proteins, we used previously developed Proteomic Screening and Identification of Amyloids (PSIA) approach. Analysis of amyloid properties of proteins was performed in Curli-Dependent Amyloid Generator (C-DAG) system, which allows to export a protein of interest to the surface of *E. coli* cells.

Results: We identified 54 proteins in detergent-resistant fraction of *Rhizobium leguminosarum* using PSIA approach. For further analysis, we chose two outer membrane proteins involved in nodulation - RopA porin, identified with the highest mass-spectroscopy score, and outer membrane protein RopB. We demonstrated that cells exporting RopA and RopB proteins exhibit typical for amyloids apple-green birefringence in polarized light upon Congo Red dye binding. Also, RopA and RopB form unbranched fibrils on the cell surface. Moreover, RopA and RopB proteins fused with YFP aggregate in yeast cells. Taking together, we may conclude that RopA and RopB are able to form amyloid fibrils.

Acknowledgements: This work is supported by the Russian Science Foundation, grant No 17-16-01100.

PT279 Effect of LolA depletion on Pseudomonas aeruginosa growth, biofilm formation, drug resistance and virulence

Roberta Stefanelli[1], Alessandra Lo Sciuto[1], Shirley Genah[1], Ehud Banin[2], Francesco Imperi[3]

[1]Sapienza University of Rome, Department of Biology and Biotechnology Charles Darwin, ROMA, Italy
[2]Bar-Ilan University, Institute for Nanotechnology and Advanced Materials, Ramat Gan, Israel
[3]Roma Tre University, Department of Science, Rome, Italy

Background: The outer membrane (OM) of Gram-negative bacteria is an asymmetric lipid bilayer which contains several integral (β-barrel) and peripheral proteins (mainly lipoproteins). This structure is essential for bacterial physiology, and provides a permeability barrier to the entry of toxic compounds. By conditional mutagenesis we recently confirmed the *in vitro* essentiality of the periplasmic protein responsible for lipoprotein transport to the OM (LolA) in the human pathogen *Pseudomonas aeruginosa*. However, LolA was surprisingly found to be less important for *P. aeruginosa* infectivity in different animal models.

Objectives: To verify the effect of LolA depletion on *P. aeruginosa* phenotypes relevant to the infection and to unravel the adaptive mechanism(s) underlying *P. aeruginosa* adaptation to low LolA levels.

Methods: The effect of LolA depletion was evaluated on (i) growth in human serum, (ii) outer membrane integrity and (iii) biofilm formation. Moreover, Biolog Phenotype MicroArray was used to identify conditions able to sustain growth of LolA-depleted cells.

Results: LolA-depleted *P. aeruginosa* cells showed residual growth in human serum, and this was found to be at least partly related to the high ionic strength of this medium. High salt concentrations also partially restored drug and detergent resistance in LolA-depleted cells. Moreover, LolA-depleted cells formed mature biofilms which were however more sensitive to antibiotics. The rescue effect of salts on growth was not due to overproduction of exopolysaccharides or activation of the recently-described Cpx stress response system. Membrane fractionation experiments are ongoing to verify to what extent LolA-depleted growth affects lipoprotein distribution and/or abundance.

PT280 A multiplicity of adaptive strategies in phytopathogenic bacterium Pectobacterium atrosepticum

Olga Petrova[1], Vladimir Gorshkov[1;2], Amina Daminova[1;2], Iuliia Sergeeva[2], Natalia Gogoleva[1;2], Yuri Gogolev[1;2]

[1]Kazan Institute of Biochemistry and Biophysics, FRC Kazan Scientific Center, Russian Academy of Sciences, Kazan, Russian Federation
[2]Kazan Federal University, Kazan, Russian Federation

Background: Contemporary views on bacterial adaptation point to the existence of universal cascade of stress reactions: the induction of stringent response that activates global stress regulator RpoS. These reactions take place at high population density resulting in the formation of the particular type of resistant cell forms. However, considering a variety of stress factors, different physiological states of microorganisms and unequal population densities under stress effect, multiple adaptive programs in bacteria should exist.

Objectives: We have searched for differences in realization of stress responses under starvation in phytopathogenic microorganism *Pectobacterium atrosepticum (Pba)* to characterize alternative ways of bacterial adaptation.

Methods: Wild type and mutants of *Pba* at high (10^6-10^9) or low (10^1-10^5) population densities, and at different physiological states (exponentially growing and stationary phase cells) were exposed to carbon or nitrogen starvation and starving cultures were monitored by means of CFU and genome copy counting, electron microscopy, qPCR, RNA-Seq analysis, cross-protection tests.

Results: The following events occurred in starving *Pba* populations: cell lysis at high density or adaptive proliferation at low one, the activation of stringent response under carbon, but not nitrogen, starvation, the induction of RpoS-dependent resistance under carbon starvation or RpoS-independent tolerance under nitrogen one, the formation one or the another resistant phenotypes depending on the parameters of starvation. All starving cultures were able to persist for a long time under adverse conditions, acquired cross-protection and retained virulence indicating that alternative ways may lead to adaptation of a particular bacterial species. This study was supported by RSF (19-14-00170).

PT282 OmpA is a potential molecule involved in the interaction of Salmonella Pullorum and chicken ovarian granulosa cells

Masashi Okamura[1], Satomi Tanaka[1], Hisaya Ono[1], Dong-Liang Hu[1]

[1]*Kitasato University School of Veterinary Medicine, Laboratory of Zoonoses, Towada, Aomori, Japan*

Background: *Salmonella enterica* serovar Gallinarum biovar Pullorum (SP) causes pullorum disease in chicks, which is responsible for severe economic losses on poultry industry through the mortality of progeny after vertical (transovarian) transmission of the bacteria from infected parent stock. We have found that SP can invade the chicken ovarian granulosa cells (cGCs). Since cGCs form a layer structure adjacent to the yolk membrane, it is hypothesized that interaction of SP and cGCs is a crucial step for transovarian transmission.

Objectives: This study is to elucidate the mechanism(s) by which SP interacts with cGCs.

Methods: A bacterial molecule(s) interacting with the cGC membrane surface before invasion process was found by magnetic beads pull-down assay and identified by nano LC-MS/MS analysis. Potential involvement of the detected protein was evaluated by (1) binding of the protein to the cultured cell surface observed by using fluorescence microscopy and (2) decreased association of SP with the cultured cGCs by addition of antisera against the protein to SP prior to *in vitro* infection.

Results: One major bacterial outer membrane protein eluted after pull-down assay was identified as OmpA. Recombinant OmpA (rOmpA) was shown to bind to the cultured cGCs. Addition of anti-OmpA sera to SP prior to inoculation to cultured cGCs resulted in a significant decrease in the ratio of bacterial association to 4.39% from 12.89% shown in addition of control sera (P=0.02). These results suggest that OmpA is a potential molecule involved in the interaction of SP and cGCs.

PT283 Small molecule inhibitors of formate-nitrate-transporters from human pathogenic Plasmodium species

Anna Meier[1], Eric Beitz[1]

[1]Pharmaceutical Institute, Christian-Albrechts-University Kiel, Pharmaceutical / Medicinal Chemistry, Kiel, Germany

Background: We discovered the missing lactate transporter of Plasmodium falciparum, PfFNT. It is a member of the formate-nitrate-transporter family and vital for the parasite. First inhibitors killed cultured plasmodia at nanomolar concentrations. We were able to select a mutation resistance by sub lethal compound concentrations. This mutation originates from a single amino acid exchange. It helped us gain information about the mechanism of inhibition. No information is available on the FNTs from the other human pathogenic Plasmodium species, in particular Plasmodium vivax, regarding function and inhibitors.

Objectives: We aim at identifying novel lead structures with activity against the resistance mutant. We will also express and characterize the remaining human pathogenic Plasmodium FNTs.

Methods: FNT is expressed in Saccharomyces cerevisiae yeast lacking the endogenous monocarboxylate transporters. 13,000 compounds from the Tres Cantos Antimalarial Set, TCAMS, are tested in a phenotypic assay that has lactate as sole carbon source. For better signal to noise ratio the cell viability marker resazurin is added. To obtain rates of lactate uptake and to generate IC_{50} values for compounds, a biophysical assay with ^{14}C-labelled lactate is employed.

Results: Yeast expressing PfFNT grows on lactate media and the growth can be blocked with PfFNT inhibitors. The similarity in protein sequence of all human pathogenic Plasmodia is above 84 %. They show similar rates of lactate uptake and a comparable inhibitory potential so far.

PT284 Vancomycin and D-Cycloserine Combination in Vancomycin Resistant Staphylococcus aureus (VRSA) and Enterococci (VRE): From Synergy to Antagonism

Abdelhakim Boudrioua[1], Axel Hartke[1], Caroline Giraud[1]

[1]*Normandie Univ, UNICAEN, U2RM, 14000 Caen, France*

Background: The increasing spread of antibiotic resistant bacteria is a major human health issue. Vancomycin (VAN) is a glycopeptide antibiotic targeting D-ala-D-ala dimers during peptidoglycan biosynthesis. It is used as last resort treatment of Methicillin resistant *Staphylococcus aureus* (MRSA) and Enterococci infections. The challenging development of new effective antibiotics has led to focus on seeking synergistic combinations. In a recent paper, the combination of VAN and D-cycloserine (DCS) was found to be synergistic in VRSA strains lacking *van* operon, which is expected because DCS disrupts peptidoglycan biosynthesis by inhibiting D-ala-D-ala ligase (Ddl).

Objectives: We hypothesize that VAN-DCS combination leads to opposite effects in VRSA and VRE strains harboring *van* operon. In these strains, VAN resistance is confered by the synthesis of modified pentapeptide precursors ending in D-ala-D-lactate instead of D-ala-D-ala.

Methods: To assess the outcome of VAN-DCS combination in *van* operon lacking and *van* operon harboring strains we determined the Minimum Inhibitory Concentration of VAN and DCS, then calculated the Fractional Inhibitory Concentration index (FIC) using the checkerborard method.

Results: The calculated FIC of VAN-DCS combination in a vancomycin intermediate, *van*A-type and *van*B-type strains were 0.5, 5 and 3 respectively. As a result, VAN-DCS combination leads to synergy in *van*-lacking strains, and to antagonism in both VRSA and VRE strains harboring *van* operon. The VAN-DCS antagonism is due to a mechanism that we named *van*-mediated Ddl inhibition bypass. This study points out the importance of taking into account the genetic background of treated strains to prevent inappropriate antibiotic combinations.

PT285 The transciptome analysis of drug tolerant Mycobacterium tuberculosis persister induced by in vitro antibiotic treatment

Jae Wook Hyeon[1], Sungkyung Lee[1], Sung Soon Kim[1], Seonghan Kim[1]

[1]Korea National Institute of Health, Centers for Disease Control and Prevention, Division of Bacterial Disease Research, Cheongwon-gun, Korea, Rep. of South

Background: Tuberculosis (TB) is a globally problematic infectious disease. The lengthy treatment is a major difficulty for control of TB, and drug tolerant persistence is known to major cause of lengthy treatment. But little is known about the characteristics of *Mycobacterium tuberculosis (Mtb)* persister so far.

Objectives: We analyzed the transcriptome of *Mtb* H37Rv and K, treated with Rifampicin (RIF), Isoniazid (INH) in order to characterize the *in vitro* generated drug tolerant persister.

Methods: H37Rv and K (CP007803.1) were cultured in 7H9 medium, and antibiotics were added to generate drug tolerant persisters. Total RNA were isolated from each culture at 0, 7, 14, 21 days after antibiotic treatment. RNAseq was performed for transcriptome analysis.

Results: Total 4008 (H37Rv) and 4194 (K) genes were identified from RNAseq. In the H37Rv, 46 and 18 genes were up- or down- regulated >4-fold in antibiotics induced persisters. Those are functionally related to ion transport and metabolism. In RIF induced persister, *Rv2060* predicted to ABC transporter, and in INH induced persister, *TB31.7* involved in stress response were remarkably changed. Compared to the H37Rv, 13 and 160 genes were up- or down- regulated >4-fold in the K. Among them, 130 genes are from INH induced persister and most are hypothetical proteins. According to the differences in expression profile of persister between the strains or between the class of antibiotics, futher study on *Mtb* persister characteristics is required.

PT286 Occurrence of drug resistant mutation during the reactivation of persister in Mycobacterium tuberculosis

Jae Wook Hyeon[1], Nara Lim[1], Sung Soon Kim[1], Sungkyoung Lee[1], Seonghan Kim[1]

[1]Korea National Institute of Health, Centers for Disease Control and Prevention, Division of Bacterial Disease Research, Cheongwon-gun, Korea, Rep. of South

Background: Drug tolerant persister population is known to causing lengthy treatment of tuberculosis, and longer survial of persister can contribute to the generation of drug resistance.

Objectives: In this study, we examined the ocurrence of drug resistant mutation during the drug tolerant persister genesis, in order to investigate their relation.

Methods: *Mtb* H37Rv and K strain were cultured in 7H9 medium, and antibiotics was added to generate drug tolerant persisters. At 0, 7, 14, 21, 28days after antibiotic treatment, a sample was collected and plated for CFU count. *rpoB* and *katG* were sequenced and the MIC for Rifampicin(RIF) and Isoniazid(INH) were measured.

Results: No mutation was found in direct culture collect, but various resistant mutation was detected in CFU count plates. In H37Rv, 14 days after each treatement for RIF and INH, mutations were detected in *rpoB* and *katG*, respectively. Mutations in *rpoB* was detected in all the three times of perfomances. Mutations in *katG* was detected only in the one of three times tests. The result from the K strain was similar to the H37Rv, but the mutation frequency of *rpoB* was less than that of H37Rv. All of *rpoB* mutant have the mutation at the 'hot spot' codon, and the MIC values for RIF were increased. But, the MIC values for INH of *katG* mutants were unchanged. In this study, all the resistance mutations were detected during the reactivation of persister. Therefore further study about the relation between occurrence of resistance and the characteristics of persistence is required.

PT287 REGULATORY ROLES OF PYRUVATE-SENSING TWO-COMPONENT SYSTEM PyrSR (YpdAB) IN ESCHERICHIA COLI K-12

Yukari Miyake[1], Hiroki Watanabe[1], Kaneyoshi Yamamoto[1;2], Akira Ishihama[1;2]

[1]*Hosei University, Department of Frontier Bioscience, Tokyo, Japan*
[2]*Hosei University, Research Center for Micro-Nano Technology, Tokyo, Japan*

Background: When the rate of production of metabolites in bacteria exceeds the amounts needed for cell growth, excess metabolites are secreted into the extracellular environment. Upon entry into poor nutrient conditions, overflowed exometabolites are reused to continue cell growth and survival. At present, however, the genetic system for utilization of exometabolites is poorly understood. A two-component system (TCS) YpdAB of *E. coli* K-12 was predicted to participate in regulation of this process. The *yhjX* gene encoding the Major Facilitator Superfamily transporter with an as yet unidentified function was identified as a single regulatory target of YpdB.

Objectives: The purpose of this study was to identify the whole set of regulatory targets of TCS YpdAB.

Methods: For identification of regulatory targets of YpdB, Genomic SELEX (gSELEX) screening was performed *in vitro.* Gel shift assay *in vitro* and reporter assay *in vivo* were carried out to confirm the regulatory targets of YpdB.

Results: Using gSELEX screening *in vitro*, we identified up to eight regulatory targets of YpdB, including the hitherto known *yhjX* gene. The predicted regulatory targets were all confirmed to be under the direct control of YpdB by gel shift assay *in vitro* and reporter assay *in vivo*. After checking various metabolites, we identified the major exometabolite pyruvate as the inducer. We then propose to rename YpdAB as PyrSR (regulator of pyruvate reutilization). One unique feature of PyrSR is its cross-talk with another pyruvate-sensing BtsSR at the TCS stage-1 for fine tuning of pyruvate reutilization.

PT288 E169 in CTX-M-15 plays an imperative role in its physiological activity in gram negative bacteria

Jyoti Verma[1], Anindya Sundar Ghosh[2]

[1]*Indian Institute of Technology, Advance Technology Development Centre, Kharagpur, India*
[2]*Indian Institute of Technology, Department of Biotechnology, Kharagpur, India*

Background: Production of CTX-M type extended spectrum beta-lactamase (ESBL) in gram negative bacteria has limited the antibacterial therapeutic options. Though the functional significance of several active site residues in CTX-M have been thoroughly investigated, there are a few reports that suggest that amino acids present outside the active site may even play a crucial role in assisting in hydrolyzing antibiotics, especially those present at the Ω-loop.
Objectives: To identify the role of glutamate169 present in the Ω-loop of CTX-M-15 beta-lactamase.

Methods: CTX-M-15 gene was cloned in pBAD18cm and the glutamic acid residiue present at the 169th position within the Ω-loop was substituted with leucine (E169L) using site-directed mutagenesis. Antibiotic susceptibility of CTX-M-15 and its mutant were tested for penicillin and cephalosporin group antibiotics. Biofilm forming ability of *E. coli* CS109 cells harbouring CTX-M-15 and CTX-M-15E169L were assayed qualitatively and quantitatively. The truncated proteins (without signal peptide) were cloned in pET28a, expressed and purified for determining kinetic behaviours to understand the effect of the mutation.
Results: E169L substitution exhibits a suppressive effect on the activity of CTX-M-15 as depicted by the increase in susceptibility (by several folds) of CS109 harboring CTX-M-15E169L, especially against piperacillin. CTX-M-15 and CTX-M-15E169L expressions in CS109 reduced its ability to form biofilm. Kinetic analysis indicates a loss of catalytic efficiency in the mutated proteins than the wild-type CTX-M-15. We conclude that E169L substitution in CTX-M-15 has effectively sensitized the cells to various beta-lactam agents, indicating the importance of E169 for the hydrolyzing activity of CTX-M-15.

PT289 Effect of LPS aminoarabinosylation on colistin resistance and fitness in Pseudomonas aeruginosa

Alessandra Lo Sciuto[1], Matteo Cervoni [2], Carmine Mancone[3], Francesco Imperi[4]

[1]Sapienza University of Rome, Department of Biology and Biotechnology Charles Darwin, ROMA, Italy
[1]Sapienza University of Rome, Department of Biology and Biotechnology Charles Darwin, ROMA, Italy
[3]Sapienza University of Rome, Department of Cellular Biotechnologies and Haematology, ROMA, Italy
[4]Roma Tre University, Department of Science, Roma, Italy

Background: Colistin represents the last-line treatment option against many multi-drug resistant Gram-negative pathogens, even if reports of colistin resistance in clinical isolates are rising. Aminoarabinosylation of LPS is a common colistin resistance mechanism in Gram-negative bacteria, and represents an essential step for the acquisition of resistance in *Pseudomonas aeruginosa*. However, whether it is sufficient to confer high level of colistin resistance in this bacterium remains unclear.

Objectives: To determine the actual contribution of LPS aminoarabinosylation to colistin resistance in *P. aeruginosa*, and to evaluate the effect of this resistance mechanism on *P. aeruginosa* fitness.

Methods: Recombinant strains constitutively expressing the enzymes for LPS aminoarabinosylation were generated in a small collection of reference and clinical isolates and verified by LPS extraction and mass spectrometry. Colistin resistance was evaluated in different culture media, while fitness was assessed by monitoring growth, biofilm formation, cell envelope stability and drug resistance.

Results: The effect of LPS aminoarabinosylation on colistin resistance was found to be culture strain- and condition-dependent. Higher levels of resistance were obtained in MH II as compared to MH, indicating that divalent cations are important for aminoarabinosylation-mediated colistin resistance. High resistance was also observed in human serum and in artificial sputum for most strains, which should partly mimic growth conditions during infections. Irrespective of the extent of its effect on colistin resistance, LPS aminoarabinosylation did not cause any fitness costs *in vitro* in any strains.

PT290 Evaluation of the essentiality of iron/sulphur cluster biogenesis in Pseudomonas aeruginosa

Maria Concetta Spinnato[1], Alessandra Lo Sciuto[2], Shirley Genah[2], Ehud Banin[3], Frederic Barras[4], Francesco Imperi[1;2]

[1]*Roma Tre University, Department of Science, Rome, Italy*
[2]*Sapienza University of Rome, Department of Biology and Biotechnology Charles Darwin, ROMA, Italy*
[3]*Bar-Ilan University, Institute for Nanotechnology and Advanced Materials, Ramat Gan, Israel*
[4]*Institut Pasteur, Department of Microbiology, Paris, France*

Background: Iron/sulphur (Fe/S) clusters are essential cofactors of proteins involved in multiple cellular processes. Most studies about the functioning and role of Fe/S biogenesis in bacteria have been conducted in the model organism *Escherichia coli*, which contains two Fe/S biogenesis systems, namely ISC and SUF. However, some important human pathogens only possess a single Fe/S biogenesis system, either SUF (e.g., *Mycobacterium tuberculosis*) or ISC (e.g., *Pseudomonas aeruginosa*).

Objectives: To characterize the effect of impaired Fe/S biogenesis in *P. aeruginosa* and to validate this process as a potential antibacterial drug target.

Methods: A conditional mutant in which the expression of a key ISC protein (IscU) is under the control of an arabinose-dependent promoter was generated in the reference strain *P. aeruginosa* PAO1. Planktonic and colony growth was assessed by standard methods, under both aerobic and anaerobic conditions. Biofilm formation was investigated with microtiter plate and flow chamber biofilm assays. Antibiotic resistance was monitored by disc diffusion test, MIC and time-kill assays. Pathogenicity was evaluated in the *Galleria mellonella* infection model.

Results: We found that IscU-depleted *P. aeruginosa* cells are unable to grow under both aerobic and anaerobic conditions, and are also defective in biofilm formation. Growth arrest due to IscU depletion is related to a marked reduction in the activity of selected Fe/S enzymes. Differently from what observed in *E. coli*, Fe/S clusters-depleted cells showed only marginal alterations in antibiotic resistance. Experiments are in progress to evaluate the effect of IscU depletion on *in vivo* growth and infectivity.

PT291 Probiotic Adhesion Properties and Beyond

Manuela Pillinger[1], Barbara Weber[1], Jutta Kesselring[1], Michaela Mohnl[1]

[1]*BIOMIN Holding, BIOMIN Research Center, Tulln, Austria*

Background: One feature assigned to probiotics is the ability to physically prevent access of harmful bacteria to their target host cells. However, adhesion/attachment per se is also associated with the infection process.

Objectives: Thus, the aim of this study was to i) develop a flow cytometer based method to determine binding efficiency of probiotics to eukaryotic cells, and ii) to determine the effect of binding on the inflammatory response.

Methods: Probiotic strains, namely *Enterococcus faecium* (EF)*, Lactobacillus reuteri* (LR), *Pediococcus acidilactici* (PA)*, Paracoccus pantothrophus* (PP)*,* and *Bacillus subtilis* (BS), were labelled with cell tracker green and incubated with host cells. Non-adhering bacteria were washed off, followed by fixation of cells and flow cytometric analysis. As only bacterial cells were stained, the mean fluorescence intensity allowed to estimate the number of bacterial cells per host cell.

Results: The number of bound bacterial cells differed depending on species, but was not affected by host cell type (gill cells versus primary intestinal epithelial cells). For example*,* on average 100 *L. reuteri* cells adhered per host cell, whereas the number of adherent *P. acidilactici* cells was 10 times lower. The interaction was specific as heat inactivated cells lost adherence. Interleukins were analyzed to estimate the impact of adhesion on the host cell. An inverse correlation of pro-inflammatory interleukin 8 production to adherence of the same probiotic was observed. Binding of *L. reuteri* did not greatly antagonize IL-8 production in LPS-stimulated host cells, whereas *P. acidilactici* significantly reduced IL-8 secretion in the same cells.

PT292 A Polar Landmark Protein Recruits The Flagella Assembly Protein FapA Under Glucose Limitation In Vibrio vulnificus

Yeong-Jae Seok[1], Yeon-Ran Kim[1], Soyoung Park[1], Chang-Ro Lee[2], Kyu-Ho Lee[3]

[1]Seoul National University, School of Biological Sciences and Institute of Microbiology, Korea, Rep. of South
[2]Myongji University, Yongin-si, Korea, Rep. of South
[3]Sogang University, Korea, Rep. of South

Background: How motile bacteria recognize their environment and decide whether to stay or navigate toward a more favorable location is a fundamental issue in survival. The flagellum is an elaborate molecular device responsible for bacterial locomotion, and the flagellum-driven motility allows bacteria to move themselves to the appropriate location at the right time. Our previous study identified FapA (flagellar assembly protein A) as a glucose-responsive determinant of the polar localization and assembly of the single flagellum in *Vibrio vulnificus*.

Objectives: It still remains unknown how FapA localizes to the cell poles and regulates flagellation and motility. Here, we tried to identify a polar component responsible for the localization of FapA to the flagellated pole and determining the flagellation pattern of the cell.

Methods: We performed a ligand fishing experiment to identify a factor(s) that contribute to the polar targeting of FapA and found that the polar landmark protein HubP is the determinant of the polar localization of FapA. Using motility assay in soft agar plates and transmission electron microscopy, we figured out how the spatiotemporal regulation of polar FapA localization and thereby flagellar assembly can be achieved depending on the availability of glucose.

Results: We identified (1) HubP as a modulator of polar flagellation that anchors the flagellar assembly protein FapA to the cell poles, (2) established the molecular mechanism underlying the intimate relationship between nutrient availability and flagellum-driven motility, and (3) characterized FapA as a non-ParA-like client of HubP in *Vibrio*, for the first time.

PT293 Confirmation of blaNDM-1 gene among the carbapenem resistant isolates from patients with meningitis symptoms attending a tertiary care hospital of central Nepal

Binod Rayamajhee[1], Laxman Ghimire [2], J. P. Yadav[3]

[1]Kathmandu Research Institute for Biological Sciences , Infectious Diseases and Immunology , Lalitpur, Nepal
[2]National Path Lab , Molecular Microbiology , Butwal , Nepal
[3]Patan Academy of Health Sciences, Pathology , Patan , Nepal

Background: Bacterial meningitis is being a cardinal cause of mortality and long-term neurological infections mainly in resource limited countries like Nepal. Globally growing antibiotic resistance has further worsen the burden.

Objectives: This study was designed with an aim to appraise the bacteriological spectrum from suspected meningitis cases and to confirm the blaNDM-1 gene in carbapenem resistant bacteria.

Methods: Cross sectional descriptive study was carried out from March to November, 2018. A total of 209 cerebrospinal fluid (CSF) specimens were collected from suspected meningitis patients. Collected specimens were subjected for culture, Gram stain, latex agglutination (LA) test and series of biochemical tests. Minimum inhibitory concentration (MIC) values of commonly prescribed antibiotics for identified bacteria were determined by following broth dilution technique as per the guidelines issued by the Clinical Laboratory Standard Institute (CLSI). Carbapenemase producer Gram negative rods were identified by Modified Hodge test (MHT). Quantitative PCR (Q-PCR) was performed for the confirmation of blaNDM-1 gene among the carbapenem resistant isolates.

Results: Among 209 analysed CFS specimens, 10.5% (22/209) bacterail meningitis was confirmed by culture and Gram staining while LA test confirmed 8.1% (17/209). Isolates were more Gram negative (66%) than positive (34%). More bacteria were reported from pediatric than adult patients. Haemophilus influenzae was the leading isolate (22.4%) followed by Escherichia coli (18.6%). Among the isolates, 55.2% were multidrug resistant (MDR), where PCR analysis of MHT positive isolates showed 40% of E. coli (2/5), 33.3% of Acinetobacter baumannii (1/3), and 25% of Klebsiella pneumoniae (1/4) were blaNDM-1 gene producing bacteria.

PT294 ClpX and ClpP are required for phage induction in Staphylococcus aureus

Mohammed Thabet[1], Carmen Ferrera Valle[1], Andreas Haag[1], Jose Penades[1]

[1]G12 8TA, Institute of Infection, Immunity and Inflammation, College of Medical, Veterinary and Life Sciences, Glasgow, United Kingdom

Background: Staphylococcal phages are maintained in a repressed state within a bacterial chromosome by a LexA-like repressor. LexA, the key regulator of the bacterial SOS response, has been shown to require Clp-proteases to relieve repression of its target genes by degrading its N-terminal DNA-binding domain (NTD) in *E. coli.*

Objectives: Investigate the role of Clp-proteases in *S. aureus* phage biology.

Methods: We have constructed insertional and deletion mutants of the *S. aureus* Clp-proteases and investigated in their role in phage infection and induction using titration, reporter and biochemical assays.

Results: We show that ClpP and ClpX are required for phage induction but not for phage infection. We confirmed that the NTD of C_I was sufficient to repress phage replication in the absence of *clp*P and *clp*X using reporter assays. Western blot analysis showed that the C_I repressor was processed into two fragments in both WT and mutant strains and that the NTD was maintained within the *clp*X and *clp*P mutants while it was degraded in the WT strain. Absence of *clp*P or *clp*X affected phage excision and replication to different extents. While we observed no excision and replication of the phage in the *clp*X mutant, these processes were considerably delayed and overall reduced in the *clpP* mutant. These data showed that the ClpP and ClpX proteases are involved in the phage replication cycle in *S. aureus* and that, similar to LexA in *E. coli*, these proteases are required for the removal of the C_I-NTD to relieve repression.

PT295 Characterisation of putative type IV toxin-antitoxin systems from Mycobacterium tuberculosis

Ben Usher[1], Izaak N. Beck[1], Tim R. Blower[1]

[1]*Durham University, Department of Biosciences, Durham, United Kingdom*

Background: Tuberculosis remains one of the world's deadliest diseases, responsible for 1.6 million deaths in 2017. Caused by the bacterial pathogen *Mycobacterium tuberculosis* (*M. tb*), the disease is characterised by chronic infections and increasing multi-drug resistance. Toxin-antitoxin (TA) systems are two-component stress-response systems which control bacterial growth by interfering with essential cellular processes. They are ubiquitous in bacteria, with at least eighty identified in *M*. *tb*, and have come under increased scrutiny for their potential use in antimicrobial therapy.

Objectives: This study aims to characterise three homologous putative type IV TA systems identified in *M*. *tb*: Rv0837c-Rv0836c, Rv1044-Rv1045, and Rv2827c-Rv2826c. All have been found to be upregulated during macrophage infection.

Methods: We phenotypically characterised all three systems using growth assays to demonstrate TA functionality. Antitoxin-mediated auto-regulation was assessed through β-galactosidase promoter activity assays. To elucidate the mechanism of growth inhibition, microscopy was performed in conjunction with cell-free expression assays, whilst X-ray crystallographic studies were performed to determine toxin protein structure.

Results: Our results showed that Rv1044-Rv1045 and Rv2827c-Rv2826c function as TA systems in *Escherichia coli* through a reversible bacteriostatic mechanism. Antitoxins were shown to mediate autoregulation of cognate and non-cognate TA promoters, indicating the potential for cross-regulation between systems. Evidence highlighted tRNAs as potential toxin targets, suggesting translation interference as a proposed mechanism of growth inhibiton. Furthermore, the three-dimensional structure of the Rv1045 toxin was determined, providing further insight into toxin function.

PT296 A comparison of the intestinal microbiome and metabolome of Anoplocephala perfoliata (tapeworm) infected and non-infected horses: a pilot study

Rachael Hough[1], Alessandra Frau[1], John Kenny[2], Jane Hodgkinson[3], Debra Archer[3], Chris Probert[1]

[1]*University of Liverpool, Institute of Translational Medicine*
[2]*University of Liverpool, Centre for Genomic Research*
[3]*University of Liverpool, Institute of Infection and Global Health*

Background: *Anoplocephala perfoliata* is the most pathogenic equine tapeworm. There is evidence that parasites and intestinal microbiota interact. The microbiome of tapeworm infected and non-infected horses has not been compared previously.

Objectives: To compare the colonic microbiome and metabolome (functional microbiome) of horses infected with *A. perfoliata* (TP) and non-infected controls (CO).

Methods: Diagnosis of TP was confirmed by gold standard (counting of tapeworms within the gut). Colon contents were collected from 48 horses (TP, 1+ worms, n=20, CO n=28) post-mortem at an abattoir. Faecal egg counts (FEC) were performed on rectal contents to control for other gastrointestinal parasites. The volatile organic compound (VOC) metabolome of colon contents was characterised by headspace solid-phase microextraction coupled to GC-MS. Sequencing was performed using Illumina MiSeq platform targeting the 16S rRNA gene (V4 region).

Results: Gut microbiota diversity was similar between TP and CO. Differential analyses using the DeSeq2 R package revealed a reduction in some commensal bacteria (Bacteroidales UCG-001 and Ruminococcaceae UCG-004) in TP. This may be a precursor or consequence of parasite infection. Furthermore, horses with clinically important burdens (21+ worms, n=9) had increased *Selenomonas 3*. Tapeworm status (21+ worms vs 0) described a considerable amount of variation (8%, p=0.02, permutational multivariate analysis of variance) in VOC data, FEC was not significant. Modest differences in the colonic metabolome and microbiome were observed betwen TP and CO, warranting investigations to control extrinsic factors e.g. diet and gastrointestinal disease. Further analysis will integrate VOC and microbiome data using the MixOmics R package.

PT297 1,3 diamino-7H-Pyrrol-[3,2-f] quinazolines: a potential antibacterial agent against MDR Gram-negative bacteria by targeting DHFR

Xinyi Yang[1], Guoqing Li[1], Xuefu You[1]

[1]*Institute of Medicinal Biotechnology, Pharmacology, Beijing, China*

Background: Based on previous studies, we continued our efforts to developing novel DHFR inhibitors, by combination of computer-aided drug design with virtual screening, a series of quinazolines were designed and synthesized.

Objectives: To evaluate the *in vitro*/in vivo antibacterial activity and to verify the target of quinazolines against Gram-negative (G-) bacterial strains.

Methods: A series of quinazolines were synthesized and a preliminary screening of their activity against ATCC strains of G- bacterial species was done. Compounds that displayed higher antibacterial activity than the control trimethoprim (TMP) were further evaluated against clinical strains. the mouse model of septicemia was used to assess the in vivo antibacterial activity of two compounds and assays such as enzyme inhibition and surface plasmon resonance (SPR) were used to verify DHFR as the target of the compounds.

Results: Dozens of quinazoline compounds were synthesized and prepared. Most of them show much higher in vitro antibacterial activity than TMP for G- strains. Among them, three compounds own significant activity against multi-drug resistant G- bacteria such as ESBL-producing Enterobacteriaceae strains with MIC values ranging from 0.002-2 μg/mL, while MICs of TMP ranged from 1-512 μg/mL. The mouse model demonstrated that a single intravenous administration of the compounds significantly reduces bacterial counts in the blood. The in vitro enzyme inhibition assay and the SPR analysis shown that IC50s of the compounds for DHFR ranged from 2.33-97.65 nM with affinity KD values ranging from 1.57±1.63-75.2±26.4 nM.

PT298 Role and regulation of the mqsRA toxin-antitoxin system

Clothilde Rousseau[1], Nathan Fraikin[1], Laurence Van Melderen[1]

[1]*Université Libre de Bruxelles, Cellular and Molecular Microbiology, Gosselies, Belgium*

Background: The *mqsRA* operon is a non-canonical type II toxin-antitoxin (TA) system in which the gene encoding the MqsR toxin is located upstream of that encoding the MqsA antitoxin. This system was described as a global regulator of stress response and biofilm formation in *Escherichia coli*.

Objectives: Our goal was to assess the activation of the *mqsRA* toxin-antitoxin system in response to stress and in biofilms, as well as its involvement in this process. We also aimed to study two uncharacterized promoters driving the transcription of *mqsA* independently of *mqsR*.

Methods: Using transcriptional fluorescent reporter, transcriptional activity of *mqsA* putative promoters and the promoter of the whole operon was assessed by flow cytometry under various stress conditions. Antitoxin MqsA degradation was measured by spectinomycin-chase experiments. Regarding biofilm formation, the macrocolony model was used to investigate *mqsRA* impact on this process. The transcriptional activity of the three *mqsRA* promoters was assessed in macrocolony biofilms and in liquid cultures.

Results: Both *mqsA* promoters showed transcriptional activity and, interestingly, their activity was higher than the promoter of the *mqsRA* system. On the contrary to what was previously published, we were unable to find conditions in which the TA promoters were activated or the MqsA antitoxin was unstable. Accordingly, macrocolony formation was not affected by the whole system deletion or *mqsA* overexpression. Our data therefore raise question on how the *mqsRA* system is activated and whether it contributes to stress tolerance.

PT299 In vivo induced operons in Enterococcus faecalis involved in metabolism of beta-1,6 polysaccharides and derivatives

Maxime Grand[1], Marion Aubourg[1], Andreas Pikis[2;3], John Thompson[2], Josef Deutscher[4;5], Axel Hartke[1], Nicolas Sauvageot[1]

[1]Normandie Univ, UNICAEN, U2RM, 14000 CAEN, FRANCE
[2]Center for Drug Evaluation and Research, Food and Drug Administration, Silver Spring, Maryland 20993, USA
[3]Microbial Biochemistry and Genetics Unit, Laboratory of Cell and Developmental Biology, NIDCR, National Institutes of Health, Bethesda, MD 20892, USA.
[4]Micalis Institute, INRA, AgroParisTech, Université Paris-Saclay, 78350 Jouy-en-Josas, France.
[5]Centre National de la Recherche Scientifique, UMR8261 (affiliated with the Univ. Paris Diderot, Sorbonne Paris Cité), Expression Génétique Microbienne, Institut de Biologie Physico-Chimique, 75005 Paris, France.

Background: Enterococci are widely distributed gram-positive bacteria, members of healthy human microbiota, they conversely represent a leading cause in hospital-acquired infections worldwide. The bicistronic *celBA* operon supposed to be involved in metabolism of the β-1,4-linked diholoside cellobiose in the opportunistic pathogen *Enterococcus faecalis* has been shown to be induced during intraperitoneal infection conditions in mice by RNA-seq analysis.

Objectives: The characterization of this operon may lead to a better understanding of the link between carbohydrate metabolism and the virulence of *Enterococcus faecalis*.

Methods: Genes expression was assayed using RT-qPCR and direct interactions between proteins and DNA were analysed by Electrophoretic Mobility Shift Assays (EMSA).

Results: Here we show that the previously named *celBA* operon is in fact involved in transport and catabolism of the β-1,6-linked gentiobiose instead of the predicted cellobiose and is also crucial to metabolise amygdalin, a cyanogenic glycoside derived from gentiobiose. These genes encoding a PhosphoTransferase System (PTS) and 6-phospho-β-glucosidase were consequently renamed *genB* and *genA* respectively. The GenR transcriptional regulator belonging to RpiR family and encoded by the gene located upstream of *genB* gene is the major regulator of this system. This regulator can bind the *genB* promoter and act as an activator on expression of the *genBA* operon in presence of the inductor gentiobiose-6'-phosphate. The *genBA* operon is also repressed by the catabolite response regulator CcpA in presence of glucose. Interestingly, GenR seems to be able to allow induction of other operons involved in different carbon sources like maltose and maltodextrin or still uncharacterized operons.

PT300 exploring the quorum sensing system plcr-papr in bacillus cereus group

Avishag Yehuda[1], Leyla Slamti[2], Didier Lereclus[2], Zvi Hayouka[1]

[1]*The Hebrew University of Jerusalem, The Robert H. Smith Faculty of Agriculture, Food and Environment, Rehovot, Israel*
[2]*Inra, Jouy-en-Josas, France*

Background: Bacterial cell-cell communication, termed quorum sensing (QS), regulates important adaptive activities such virulence, antibiotic production, sporulation and competence, according to population densities. Developing methods to manipulate bacterial quorum sensing systems have attracted significant interest as a potential strategy for attenuating infection.
Bacillus cereus, an opportunistic human pathogen which is a member of the Gram-positive B-bacilli group that includes *Bacillus thuringiensis,* an insect pathogen, and *Bacillus anthracis*, utilizes autoinducing PapR peptide signals to mediate QS by activating pleiotropic virulence regulator PlcR.

Objectives: As the blockade of quorum sensing offers a novel strategy for attenuating bacteria pathogenic functions, we studied the structure and molecular activity of the PlcR – PapR QS system in *B. cereus.*

Methods: Based on biochemical and structural findings, we designed, synthesized and characterized direct QS synthetic PapR 7-mer derived peptides to determine the functionally critical residues within PlcR – $PapR_7$ interactions.

Results: Our finding reveals a set of non-native peptides that can inhibit PlcR regulon activity and relevant virulence factor as hemolysis activity with micromolar IC_{50} values. Moreover, we could demonstrate that the inhibition is mediated by quorum sensing and does not affect bacterial growth. We are currently studying the mode of action of these designed inhibitors, to better understand if they act inside the bacterial cells (via interaction with PlcR) or extracellulary (via binding interference with the import system). To the best of our knowledge, these peptides represent the first potent synthetic inhibitors of QS in *B. cereus* reported and have potential for use as novel antimicrobial compounds.

PT301 Dynamic genetic adaptation in the bacterium Cupriavidus metallidurans in response to uranium

Rogiers Tom[1;2], Williamson Adam[2], Rob Van Houdt[1], Natalie Leys[1], Nico Boon[2], Kristel Mijnendonckx[1]

[1]*SCK•CEN, Microbiology, Mol, Belgium*
[2]*UGent, Center for Microbial Ecology and Technology, Gent, Belgium*

Background: The bacterium *Cupriavidus metallidurans* is a well-known model organism for metal resistance and is mostly isolated from industrial sites linked to mining, metallurgic and chemical industries. The interaction of *C. metallidurans* with uranium (U^{238}) and americium (Am^{241}) has been demonstrated, however, neither the genes or proteins involved nor the precise mechanism is known.

Objectives: The goal of this project is to unravel the molecular uranium resistance mechanisms in the strain *Cupriavidus metallidurans* NA4.

Methods: Via a laboratory evolution experiment, a spontaneous *C. metallidurans* NA4 mutant (NA4U) was obtained. Whole genome sequencing and RNA sequencing were performed on NA4U to identify mutations concerning the enhanced uranium resistance phenotype together with differentially expressed genes. The interaction of NA4U with uranium was investigated by growing NA4U in presence of 340 µM $UO_2(NO_3)_2$ and measuring the amount of uranium in the supernatant and cell pellet via ICP-MS.

Results: The minimal inhibitory concentration of $UO_2(NO_3)_2$ for the parental NA4 strain is 125 µM while the uranium mutant is able to resist up until 1mM of $UO_2(NO_3)_2$. Whole genome sequencing revealed a 999 bp deletion in *czcS2*, coding for a sensor histidine kinase of the two-component system *czcR2S2.* This mutation is probably causing the constitutive over-expression of the response regulator (CzcR2) in NA4U compared to the parental strain, which in turn is causing the uranium resistance phenotype. Furthermore, growth of NA4U in presence of 340 µM $UO_2(NO_3)_2$ gave preliminary evidence that NA4U is using an active mechanism to retain uranium from the medium.

PT302 Developing a molecular bacterial load assay (MBLA) for Mycobacterium abscessus

Daniela Alferes de Lima[1], Pauline Claxton[2], Ian Laurenson[2], Devesh Dhasmana[1;3], Stephen Henry Gillespie[1], Derek Sloan[1]

[1]*University of St Andrews, School of medicine, St Andrews, United Kingdom*
[2]*NHS Lothian, Scottish Mycobacteria Reference Laboratory, Edinburgh, United Kingdom*
[3]*NHS Fife, Victoria Hospital, Kirkcaldy, United Kingdom*

Background: *Mycobacterium abscessus* is the most common rapid growing (RGM) non-tuberculous mycobacteria (NTM) causing NTM pulmonary disease (PD). Treatment involves long and toxic multi-drug regimens with uncertain benefit. Current treatment monitoring includes semi-quantitative culture of serial clinical samples that takes weeks to provide results.

We have developed an assay, the molecular bacterial load assay (MBLA) for *M. abscessus*. The MBLA targets the species specific portion of 16S ribosomal RNA region using real-time reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) aiming to accurately and rapidly quantify the viable bacterial load from patients' sputum samples.

Objectives: To validate *M. abscessus* MBLA as a treatment monitoring tool for *M. abscessus*.

Methods: Primer sensitivity was tested using serial dilutions of RNA extractions alongside CFU plates. RT-qPCR optimisation was performed through comparing serial dilutions of the same sample under different conditions. Specificity was tested through RT-qPCR of DNA of other common pulmonary pathogens. Patient samples from NHS Scotland are under evaluation currently.

Results: The MBLA shows a detection range of 10 to $\geq 1x10^7$ CFU/ml. Following RT-qPCR optimisation, the reaction efficiency is above 95%. It did not detect any of the other common pulmonary pathogens tested.

M. abscessus MBLA has high sensitivity and specificity. The RT-qPCR efficiency is within the desired range of 90% to 110%. The *M. abscessus* MBLA is the first quantitative treatment monitoring tool under development for NTMs, with added advantages of not requiring decontamination and shorter time to results.

PT303 The Effects of Human Urine on Growth and Gene Expressions in UPEC

Fatma Kal ayci Yüksek[1], Defne Gümüş[1], Özlem Sefer[2], Emre Yörük[2], Gülşen Uz[2], Mine Anğ Küçüker[1]

[1]*İstanbul Yeni Yüzyıl Üniversitesi, Medical Faculty, Medical Microbiology, Turkey*
[2]*İstanbul Yeni Yüzyıl Üniversitesi, Molecular Biology and Genetics, Turkey*

Background: The association between environmental factors and bacterial biological processes are well known. Different body fluids may also affect bacterial traits. Urine has been selected to provide host conditions which thought to influence both the growth and gene expression of bacteria.

Objectives: We aimed to investigate whether the presence of urine (naturel habitat of UPEC) influences the growth and expressions of sfa/foc (fimbrial adhesions SF1C), cnf 1 (cytotoxic necrotizing factor 1), and usp (a uropathogenic-specific protein), aer (aerobactin) genes in UPEC (C7 and C149) strains.

Methods: Healthy male urine was used for culturing of bacteria. Standard American Petroleum Institute (SAPI) medium supplemented with 30% (v/v) adult bovine serum was used as control. Bacteria were incubated at 37°C and growths were determined by measuring the changes in absorbance at 600 nm in four-, six- and 24-hour periods. Gene expression levels were determined by q-PCR. Statistical analysis was performed via Tukey's post hoc-test.

Results: Urine was found to be statistically increased the growth of both C7 and C149 ($p<0.0001$) in 4th, 6th and 24th hours of incubation. The expression levels of cnf 1, sfa/foc, and usp genes of C7 strain were found to be not affected ($p> 0.05$); the expression level of aer in C149 strain was shown to be down regulated ($p< 0.001$). This study has suggested that, urine affected both the growth and gene expression in UPEC. Therefore, modulation of virulence traits were not needed when the growth of bacteria were enhanced effectively.

PT304 Studies on PA2121 - a LysR-type transcriptional regulator from Pseudomonas aeruginosa

Magdalena Modrzejewska[1], Adam Kawałek[1], Aneta Agnieszka Bartosik[1]

[1]*Institute of Biochemistry and Biophysics, Department of Microbial Biochemistry, Warsaw, Poland*

Background: *Pseudomonas aeruginosa*, a facultative human pathogen causing nosocomial infections, has complex regulatory systems involving many transcriptional regulators. The LTTR family (LysR-Type Transcriptional Regulators) include proteins involved in regulation of various processes including stress response, motility, virulence or amino acid metabolism.

Objectives: The aim of this study was characterization of the LysR-type regulator PA2121 from *P. aeruginosa*.

Methods: *P. aeruginosa* PAO1161 strain overproducing PA2121 was obtained by introduction of the expression vector with *pa2121* gene under control of inducible *araBAD*p promoter. RNA-seq, qPCR, and electrophoretic mobility shift (EMSA) assays with recombinant protein were applied in functional characteristics of PA2121.

Results: Purified His_6-PA2121 specifically bound to the DNA preceeding the gene in *P. aeruginosa* genome, suggesting presence of autoregulation. Overproduction of PA2121 led to growth inhibition of *E. coli* and *P. aeruginosa* cells. This effect was dependent on the presence of functional Helix-Turn-Helix motif, responsible for DNA binding. A mild excess of PA2121 in PAO1161 altered the expression of 148 genes (81 up- and 67 down-regulated, fold change ≥ 2). Up-regulated genes were mostly involved in protein secretion/export systems, and amino acid metabolism. Decreased expression was observed for several genes encoding proteins engaged in central intermediary metabolism. Highest down-regulation occured for *mqoA*, encoding a malate:quinone oxidoreductase (TCA cycle) and *acsA* encoding an acetyl-coenzyme A synthetase.

Overall, the presented data suggests that PA2121 may act as a transcriptional regulator engaged in modulation of vital metabolic pathways.

This work was supported by the National Science Centre in Poland (grant 2015/18/E/NZ2/00675).

PT305 DksA-dependent regulation of virulence and biofilm formation in Pseudomonas aeruginosa

Alessandra Fortuna[1], Giulia Giallonardi[1], Francesca D'Angelo[1], Paolo Visca[1], Giordano Rampioni[1], Livia Leoni[1]

[1]*University Roma Tre, Science, Rome, Italy*

Background: The stringent response regulator DksA affects virulence in several bacterial pathogens. The animal and plant pathogen *Pseudomonas aeruginosa* has two DksA paralogs: DksA1 is constitutively expressed and contains a Zn-finger motif; DksA2 does not contain Zn and it is expressed only under Zn starvation condition. The current model predicts that DksA2 might complement DksA1 function under Zn-starvation, a condition frequently found at the infection site.

Objectives: Studying the role of DksA1 and DksA2 in *P. aeruginosa* virulence.

Methods: *P. aeruginosa* single and double deletion mutants in *dksA1* and *dksA2* genes were obtained and characterized for their impact on: *i) P. aeruginosa* virulence, *in vitro* and in acute plant and animal infection models; *ii)* biofilm formation in different experimental models; *iii)* transcription and translation of an array of virulence and biofilm-related genes (by means of promoter-probe plasmids).

Results: Our analysis shows that DksA1 positively regulates the production of the three main *P. aeruginosa* quorum sensing (QS) signal molecules, and of QS-dependent secreted virulence factors, essential during the acute infection. Accordingly, *dksA1* deletion reduces *P. aeruginosa* virulence *in vivo*. On the other hand, DksA1 negatively affects biofilm formation, a phenotype related to the chronic infection. Hence, DksA could be a regulatory node contributing to the switch between acute and chronic infection. DksA-dependent regulation of the above phenotypes occurs mainly at the transcriptional level. *In trans* expression of *dksA1* or *dksA2* restores wild type phenotypes in the Δ*dksA1*Δ*dksA2* double mutant, supporting the hypothesis that the two DksA proteins are interchangeable.

PT306 Understanding Amt/Mep/Rh: Transporting Charge Through a Hydrophobic Pore

Gordon Williamson[1], Gaetan Dias Mirandela [2], Ulrich Zachariae [3], Arnaud Javelle[1]

[1]*University of Strathclyde, Strathclyde Institute of Pharmacy and Biomedical Science, glasgow, United Kingdom*
[2]*University of Edinburgh, Wellcome Trust Centre for Cell Biology, Edinburgh, United Kingdom*
[3]*University of Dundee, School of Life Sciences, Dundee, United Kingdom*

Background: Ammonium movement across biological membranes, a fundamental process in all living organisms, is mediated by the ubiquitous Amt/Mep/Rh family of membrane transporters. Ammonium is a vital nitrogen source for bacteria, fungi, and plants and a toxic metabolic waste for animals. The first genes encoding ammonium transporters were identified over 20 years ago in *Saccharomyces cerevisiae* (Mep) and *Arabidopsis thaliana* (Amt. In later years, it was shown that the rhesus protein (Rh) is an Amt/Mep ortholog in vertebrates and since then, members of the Amt/Mep/Rh protein family have been identified in almost all sequenced organisms. The physiological importance of this family is underlined by the role of Mep2 in filamentation, a dimorphic transition related to the virulence of pathogenic yeast. Despite 20 years of research, the exact mechanism of Amt/Mep/Rh remains elusive. The crystal structure of AmtB suggested that transport was **electroneutral**, however functional information demonstrates that transport is **electrogenic**.

Objectives: To reconcile the confliciting functional and structural information and propose a mechansitic model for Amt/Mep/Rh mediated ammonium transport using the *E. coli* ammonium transporter AmtB.

Methods: Molecular Dyanmic Simulations (MDS) were used to predict the impact of amino acid substitions on AmtB.

Solid-Supported Membrane Electrophysiology (SSME) was used to characterise the transport activity and selectivity of WT and variant AmtB.

Results: MDS revealed the presence of two water wires within AmtB. SSME demonstrated that these wires are vital for AmtB-mediated transport. The findings suggest that AmtB functions as an NH_3/H^+ symporter.

PT307 Sistematic mutational analysis reveals the diguanylate cyclases involved in the alginate biosynthesis by Azotobacter vinelandii

Carlos Ahumada[1;1], Josefina Guzman Aparicio[1], Brian Hsueh[2], Christopher Waters[2], Guadalupe Espin[1], Cinthia Nuñez[1]

[1]*Instituto de Biotecnología Unam, Cuernavaca, Mexico*
[2]*Michigan State University, East Lansing, United States*

Background: *Azotobacter vinelandii* is a nitrogen-fixing soil bacterium that produces the exo-polysaccharide alginate. A previous report demonstrated that the absence of MucG, a protein with putative domains for the synthesis (GGDEF) and degradation (EAL) of the second messenger bis-(3'-5')-cyclic dimeric guanosine monophosphate (c-di-GMP), negatively affected alginate synthesis. In *Pseudomonas aeruginosa* this second messenger is essential for alginate polymerization and MucR was identified as the diguanylate cyclase (DGC) necessary for the synthesis of this polymer. However, in *A. vinelandii* deletion of the *mucR* orthologue gene did not affect alginate synthesis. Besides, the genome of *A. vinelandii* encodes 24 proteins involved in c-di-GMP metabolism.

Objectives: To demonstrate the effect of c-di-GMP in alginate biosynthesis and to identify the DGC`s and phosphodiesterases (PDE) involved in alginate biosynthesis by *A. vinelandii.*

Methods: *A. vinelandii* srains with artificially elevated and reduced levels of c-di-GMP were generated. In addition, a systematic mutational analysis of the genes for c-di-GMP metabolism was conducted.

Results: Constitutive expression of a DGC or a PDE resulted in elevated or reduced levels, respectively, of c-di-GMP and the levels of this second messenger correlated with alginate production. On the other hand, two genes encoding active DGC positively controlled alginate production as their individual deletion reduced about 70% alginate production when compared with the wild type strain. These results clearly showed that in *A. vinelandii* the c-di-GMP is a positive effector of alginate production but the modular architecture of the proteins controlling this c-di-GMP pool differs from those identified in *P. aeruginosa*.

PT308 What is the role of toxin antitoxin systems in bacterial persistence ?

Nathan Fraikin[1], Frédéric Goormaghtigh[1], Marta Putrins[2], Niilo Kaldalu[2], Tanel Tenson[2], Laurence Van Melderen[1]

[1]*Université Libre de Bruxelles, Cellular & Molecular Microbiology, Gosselies, Belgium*
[2]*University of Tartu, Institute of Technology, Tartu, Estonia*

Background: Persistence is a low-frequency phenomenon allowing a bacterial subpopulation to survive antibiotic treatment. Type II toxin-antitoxin (TA) systems were assumed to cause persister cell formation in *Escherichia coli* based on the observation that successive deletions of 10 TA (*Δ10TA*) systems decreased persistence frequency. Using a fluorescent reporter and live imaging, a small subset of cells in which the *yefM-yoeB* TA system was transcriptionnally activated was shown to enter a dormancy state and survive ampicillin treatment. However, these results have lately been subject to controversy.

Objectives: We tried to reproduce previous results using an independently constructed *Δ10TA* strain and new *yefM-yoeB* fluorescent reporter.

Methods: We quantified the survival of this deletion strain to ampicillin and ofloxacin while live cell imaging during ampicillin treatment was performed on cells carrying the newly constructed fluorescent reporter. The genomes of *Δ10TA* strains from our lab and from the controversial study were also sequenced.

Results: Our *Δ10TA* mutant does not show reduced persistence to ofloxacin or ampicillin. Whole genome sequencing showed that the controversial *Δ10TA* mutant was progressively infected with several prophages, as previously reported, which could distort antibiotic survival assays. We also showed that the *yefM-yoeB* fluorescent reporter used in the controversial study is not functional and that the authors mostly tracked autofluorescence during their live imaging assays. Using a new and experimentally validated reporter, we show that *yefM-yoeB* is not activated in persister cells. Altogether, our results confirm that TA systems are not involved in persistence in *E. coli*.

PT309 Local transcription factors and global regulators in control of hexuronate metabolism and motility in Escherichia coli: the role of effector molecules.

Uliana Shvyreva[1;2], Maria Tutukina[2;3], Anna Kaznadzey[3], Yury Purtov[1;2], Tatiana Bessonova[1;2;4], Olga N. Ozoline[2]

[1]*Institute of Protein Research RAS, Pushchino, Russian Federation*
[2]*Institute of Cell Biophysics PSCBR RAS, Pushchino, Russian Federation*
[3]*Institute for Information Transmission Problems RAS, Moscow, Russian Federation*
[4]*Moscow State University, Moscow, Russian Federation*

Background: Intergenic cAMP-independent binding sites were found even for classical regulator cAMP-CRP, suggesting its role typical for a nucleoid protein. From the other side, some of the nucleoid-associated proteins that had been thought to fold the bacterial chromosome, could also participate in targeted transcription regulation.

Objectives: The aim was to reveal how the interplay between the "local" transcription factors UxuR, and ExuR, global regulator cAMP-CRP, and nucleoid protein Dps, helps controlling bacterial metabolism, their motility and virulence, and what is the impact of small effectors.

Methods: Binding sites were mapped by SELEX *in vitro* and ChiP-seq *in vivo*, compared with RNA-seq, and targets for cross-regulation were chosen. Ligands were searched using molecular docking and evaluated by EMSA.

Results: urprisingly, D-glucuronate, an intermediate of the Ashwell pathway controlled by UxuR and ExuR, changes the oligomeric structure of Dps and effects its binding. Comparison of transcriptomes revealed huge intersection between the Dps, UxuR, and ExuR target genes, with lots of motility and ferric uptake genes being regulated by all three regulators. At the same time, the regulons of UxuR and ExuR have a huge intersection with cAMP-CRP and become shared in the presence of D-glucuronate, reflecting heterodimer formation that was confirmed by both molecular docking and EMSA. Summarizing all the results, effectors play a key role in the DNA-protein interactions for both regulatory and structural proteins of bacterial nucleoid, adjusting bacterial metabolism and motility, and may thus be used as modulators for their virulence.
The work was supported by RSF 18-14-00322.

PT310 New insights into hydroxyectoine synthesis and its transcriptional regulation in the halophile Chromohalobacter salexigens

Montserrat Argandoña[1], Francine Piubeli[1], Rosa García-Valero[1], emilia Naranjo[1], Lourdes Martinez-Martinez[1], Manuel Salvador[2], Joaquin J Nieto[1], Carmen Vargas[1]

[1]Faculty of Pharmacy, Department of Microbiology and Parasitology, Sevilla, Spain
[2]Faculty of Health and Medical Sciences, Guildford, United Kingdom

Background: The broad salt-growing halophile *Chromohalobacter salexigens* is a natural hydroxyectoine producer, a compatible solute with a great interest in Biotechnology since it possesses superior function-preserving properties than its precursor ectoine. In *C. salexigens* the hydroxyectoine is synthesized in response to osmotic and heat stress, principally through the ectoine hydroxylase enzyme EctD. However, an *ectD* mutation does not totally suppress hydroxyectoine accumulation. A second gene (*ectE*) that codifies a putative ectoine hydroxylase was found in the genome of *C. salexigens* and a gene that codifies an AraC-type transcriptional regulator (EctZ) was located just upstream of *ectD*. Besides, our results demonstrated that RpoS contributes to the transcriptional activation of *ectD* gene at the early response.

Objectives: To elucidate new pathways and mechanisms controlling the synthesis of hydroxyectoine in *C. salexigens,* we investigated the role of the second ectoine hydroxylase (EctE) and the relevance of two regulators (EctZ and RpoS) at the exponential and stationary phase. This information might be used to design novel metabolic engineering strategies for hydroxyectoine overproduction.

Methods: *ectE*, *ectDE*, *rpoS* an *ectZ* mutant strains of *C. salexigens* were constructed and their intracellular profile of ectoine and hydroxyectoine (by LC-MS) and transcriptional pattern of *ectE* and *ectD* genes (by qPCR) were analyzed at different conditions of salinity, temperature, and phase of growth.

Results: EctE is a functional ectoine hydroxylase but less relevant than EctD for hidroxyectoine synthesis. *ectD* and *ectE* genes are controlled by RpoS and EctZ but in a different manner depending on external salinity and growth phase.

PT311 Role of intracellular L-proline accumulation in osmotic stress tolerance of human gastric pathogen Helicobacter pylori

Dan Su[1], Heinrich Jung[1]

[1]*Ludwig-Maximilians Universität München, Biozentrum, Department Biologie I, Mikrobiologie, Martinsried, Germany*

Background: As a major cause of gastroduodenal disease, *Helicobacter pylori* infects approximately half of the human population. For colonized individuals, epidemiological data indicate a synergistic effect between *H. pylori* infection and dietary sodium chloride intake. However, *H. pylori* does not contain many of the osmoprotective systems found in other bacteria such as *E. coli*. Since the amino acid proline acts as an osmoprotectant in many organisms, and deletion of genes encoding the proline transporter PutP and the proline dehydrogenase PutA renders *H. pylori* incapable of gastric colonization, we hypothesized that proline and related systems contribute to the osmotic stress tolerance of *H. pylori*.

Objectives: We aim to reveal the mechanisms underlying osmotic stress tolerance of *H. pylori*. The focus is on the role of proline and proline specific systems in osmoadaptation.

Methods: Growth of *H. pylori* wild-type, *putP* and *putA* deletion mutants was compared under control and osmotic stress conditions on GC-Agar and in Brucella Broth liquid culture. Moreover, the intracellular accumulation of proline was measured by using HPLC after derivatization with 9-fluorenylmethyloxycarbonyl chloride (FMOC). Furthermore, expression of *putA* and *putP* genes in the wild-type was investigated by qRT-PCR.

Results: Deletion of *putP* inhibited growth and reduced the survival rate of *H. pylori* under osmotic stress conditions. Overexpression *of putP* stimulated both parameters. Furthermore, osmotic stress increased the expression of *putP* and *putA*, and stimulated the accumulation of proline and glutamate in cells. The results suggest that both PutP and PutA contribute to osmotic stress tolerance of *H. pylori*.

PT312 IscR of Burkholderia multivorans plays both repressing and activating roles in transcription of isc operon for Fe-S cluster biosynthesis

Shouta Nonoyama[1], Kouhei Kishida[1], Yuji Nagata[1], Yoshiyuki Ohtsubo[1], Masataka Tsuda[1]

[1]*Tohoku University, Sendai, Japan*

Background: Fe-S cluster is an essential cofactor for various enzymes, and transcriptional regulation of the operon for biosynthesis of Fe-S cluster has remained unclear in many environmental bacteria.

Objectives: *Burkholderia multivorans* ATCC17616 was predicted to carry only one operon for putative biosynthesis of Fe-S cluster, and the first gene (*iscR*) in this *isc* operon encodes a putative transcriptional regulator. Here we investigated transcriptional regulation of this operon in ATCC17616.

Methods: qRT-PCR analysis was performed in ATCC17616 and its *iscR* deletant to measure transcriptional levels of a structural gene in the *isc* operon under different concentrations of iron. Biochemically purified IscR protein was used to investigate its binding to the promoter region of the *isc* operon and *in vitro* transcription assay.

Results: An ATCC17616 derivative whose entire *isc* operon was designed to be transcribed under the P_{BAD} promoter required arabinose for the growth, showing the essential role of this operon. Transcription of *isc* operon in ATCC17616 increased and decreased under the iron-depleted and -replete growth conditions, respectively. No such changes in the transcriptional levels were observed in the *iscR* deletant, and the transcriptional levels under both growth conditions were higher in the deletant than in the wild type. Our use of the purified IscR protein showed its (i) ability to specifically bind to the promoter region of the *isc* operon and (ii) transcriptional activation of the *isc* operon. All of these results suggested that IscR plays both repressing and activating roles in the transcription of *isc* operon in ATCC17616.

PT313 "Anthranilate peak" and changes in life style of Pseudomonas aeruginosa

Hyeon-Ji Hwang[1], Suran Kim[1], Xi-Hui Li[1], Joon-Hee Lee[1]

[1]College of Pharmacy, Pusan National University, Department of Pharmacy, busan, Korea, Rep. of South

Background: *Pseudomonas aeruginosa* produces various metabolites, such as phenazines, pyocyanin, quinolones, acyl-homoserine lactones, anthranilate, and so on, and most of them are secreted and accumulated during growth. Anthranilate is an important intermediate for the synthesis of tryptophan and *Pseudomonas* quinolone signal (PQS), and metabolized by anthranilate dioxygenase complex (*antABC* gene products) via TCA cycle. Anthranilate has been reported to cause biofilm dispersal in various bacteria.

Objectives: In order to elucidate the relation between the anthranilate production and biofilm formation of *P. aeruginosa*, we traced the change of the anthranilate level as *P. aeruginosa* grows, and investigated the time point when *P. aeruginosa* forms biofilm.

Methods: We measured the extracellular and intracellular anthranilate levels throughout long-term culture of *P. aeruginosa* by using reporter (*antAp-lacZ* fusion) and HPLC analyses. We also investigated the biofilm formation in static- and flow cell-systems throughout the long-term culture.

Results: The production and secretion of anthranilate remain very low until *P. aeruginosa* reaches stationary phase, but it begins to secrete at stationary phase and rapidly accumulate to a high level at late stationary phase. Interestingly, the level of anthranilate rapidly decreased again when the stationary phase persisted longer. We named this transient increase in anthranilate level “anthranilate peak”. This anthranilate peak was generated by *antABC* function, because the high-level accumulation of anthranilate lasted in *antABC* mutant. The biofilm analyses demonstrated that the accumulation of anthranilate destabilized the biofilm structure and the biofilm began to form only after the anthranilate peak disappears.

PT314 Campylobacter jejuni (11168) RidA (Cj1388) is required for flagellar biosynthesis and/or function

Jessica Irons[1], Diana Downs[1]

[1]*University of Georgia, Microbiology, Athens, United States*

Background: *Campylobacter jejuni* is a leading cause of diarrheal disease. The exact mechanism of *C. jejuni* infection is unknown, however, it requires motility. *C. jejuni* (11168) encodes two members of the **R**eactive **I**ntermediate **D**eaminase (Rid) superfamily, *cj1388* and *cj0327*. Previous reports suggest Cj1388 affects flagella-flagella interactions and virulence. The Rid protein superfamily is broadly conserved throughout all domains of life. RidA has primarily been studied in *Salmonella enterica* for its role in quenching 2-aminoacrylate (2AA) and preventing pyridoxal 5'-phosphate-dependent enzyme damage.

Objectives: This study was undertaken to understand the physiological role of Cj1388 in *C. jejuni* 11168. We used the well-studied RidA paradigm from *S. enterica* to probe the unique metabolic architecture of *C. jejuni* lacking *cj1388* and how it impacts motility and virulence.

Methods: Genetic and physiological approaches were taken. *In vivo* complementation of *S. enterica ridA* mutants by *cj1388* or *cj0327* expressed *in trans* was assessed. Phenotypic consequences of lacking *cj1388* or *cj0327* including motility, autoagglutination, and phage infectivity were monitored.

Results: Our data suggest the *C. jejuni* RidA homologue, Cj1388, plays a role in flagellar biosynthesis, and/or function and, as such is expected to influence virulence of the organism. Mutants lacking *cj1388* have defects in motility, autoagglutination, and phage infectivity under the conditions tested. The RidA paradigm indicates the phenotypes of the *C. jejuni cj1388* mutant are likely due to the inhibition of one or more PLP-dependent enzymes by the reactive enamine 2AA, efforts to define the source and targets of 2AA in *C. jejuni* are ongoing.

PT315 Diagnostic integration by Liquid Based Microbiology (LBM): bacteriology meets molecular testing in Carbapenemase-producing Enterobacteriaceae (CPE) screening workflow

Simone Ambretti[1], Andrea Berlingeri[1], Monica Cricca[1], Giovanna Angela Gentilomi[1], Giulia Lombardi[1], Gian Matteo Pollini[1], Claudio Foschi[1], Paolo Gaibani[1], Maria Carla Re[1]

[1] *S.Orsola-Malpighi Hospital, Microbiology Unit, Bologna, Italy*

Background: Carbapenemase-producing Enterobacteriaceae (CPE) have emerged over the last decade as major nosocomial pathogens that threaten the safe care of patients in many parts of the world. The strain primarily responsible for the CPE epidemic is KPC-producing *Klebsiella pneumoniae* causing high mortality and morbidity due to relevant reduction of active antimicrobials.

Objectives: Multi-disciplinary infection control measures, including active surveillance of CPE carriers by rectal swabs for contacts of positive hospitalized patients and for screening of high-risk patients was adopted at S.Orsola-Malpighi Hospital, Bologna. Aim of this study was the evaluation of a new approach of sample collection suitable for phenotypic standard tests and rapid molecular assay to improve sample workflow.

Methods: From July 2016 to June 2018, 1905 samples from two different wards (respiratory care and respiratory intensive care unit) have been selected for the study. Active surveillance with collection of one rectal swab (eSwab COPAN) was performed for all patients at the admission and once a week. The same rectal swab was analyzed with routine culture-based method (Chrom-Agar KPC, Vacutest ® Kima) and a real-time PCR assay (Xpert ® CARBA-R, Cepheid ®) detecting specific carbapenemase genes (KPC, VIM, NDM, IMP-1, OXA-48), within one hour.

Results: 1844/1905 samples were identified as negative while 61 swabs were identified as positive considering molecular testing (49 KPC, 7 VIM, 4NDM, 1 KPC+NDM). The culture-based method confirmed the growth of CPE in 54 samples (all K. pneumoniae strains). The sample workflow supported by eSwab, is able to speed up and simplify CPE active surveillance.

PT316 Biochemical insights into Glutamate decarboxylase from Mycobacterium tuberculosis

Fabio Giovannercole[1], John Blanchard[2], Daniela De Biase[1]

[1]*Università degli Studi di Roma la Sapienza - Facoltà di Medicina, Medico-Surgical Sciences and Biotechnologies, Latina, Italy*
[2]*Albert Einstein College of Medicine - Jack and Pearl Resnick Campus, New York, United States*

Background: *Mycobacterium tuberculosis*, the causative agent of tuberculosis, is a facultative intracellular pathogen which establishes a life-long infection by residing within macrophages and halting the phagosome-lysosome fusion. In response to IFN-γ, activated macrophages deliver the pathogen to the acidic compartment of the phagolysosome where pH fluctuates between 4.5-5.0. *M. tuberculosis* possesses a gene coding for a putative glutamate decarboxylase (*Mt*GadB), the role of which could be to overcome the acidity of the phagolysosome or, as part of the GABA-shunt, to compensate for the lack of the α-ketoglutarate dehydrogenase activity in *M. tuberculosis*.

Objectives: With the current study we investigated the biochemical properties of *Mt*GadB to unveil its physiological role in *M. tuberculosis*.

Methods: *Mt*GadB (carrying an N-terminal His_6-tag) was expressed and purified in *Escherichia coli* via affinity chromatography and its pH-dependent spectroscopic and enzymatic properties were investigated and compared with those of the extensively studied *E. coli* homologue (*Ec*GadB).

Results: We developed a successful protocol for the expression and purification of His_6-*Mt*GadB. We found that the His_6-tag negatively influences the oligomeric structure and the enzymatic activity of *Mt*GadB, in agreement with the structural role of the N-terminus in *Ec*GadB. UV-Visible and fluorescence spectroscopic studies showed that *Mt*GadB shares with the *Ec*GadB counterpart many biochemical similarities. This is in line with the full conservation of GAD-signature residues observed in *Mt*GadB. Given the possible involvement of *Mt*GadB in the GABA-shunt, this work sets the basis for a better understanding of the *M. tuberculosis* GABA-shunt enzymes.

PT317 Reduction of anaerobic electron acceptors drives biofilm formation in Shewanella algae

Jonatan Martín-Rodríguez[1], José Antonio Reyes-Darias[2], Iris Manzano[3], José Manuel González Hernández[3], Åsa Sjöling[1], Tino Krell[2], Ute Römling[1]

[1]*Karolinska Institutet, Solna, Sweden*
[2]*Estación Experimental del Zaidín, CSIC, Granada, Spain*
[3]*Universidad de La Laguna, La Laguna, Spain*

Background: *Shewanella algae* is a marine bacterium with an outstanding respiratory versatility, being able to use a plethora of electron acceptors (EAs) under anaerobic conditions. This facilitates bacterial proliferation in oxygen-depleted niches. Bacterial adhesion and biofilm formation are pivotal events in environmental substrate colonization. A strain-specific, dose-dependent association between biofilm formation and EA supplementation is observed among *S. algae* isolates.

Objectives: To explore the physiological links between reduction of EAs and biofilm formation in *S. algae*.

Methods: We have used bacterial genetics, transcriptomics and imaging to disentangle the above-mentioned physiological association using *S. algae* CECT 5071^{TS} as an archetypal *S. algae* model.

Results: Here we show, unambiguously, that reduction of EAs drives biofilm formation in *S. algae* strain-specifically. Deletion of the catalytic subunits in the relevant reductase operons results in the abolishment of enzymatic activity and loss of biofilm formation upon supplementation with the specific EA. Ectopic expression of the relevant operons restores both phenotypes. When incorporated into experimental abiotic substrata, increased surface colonization by strains responding to EA addition with increased biofilm production is observed. The strain-specific nature of this phenomenon likely involves the participation of specific signal transduction pathways as suggested by the transcriptomic analysis of the type strain exposed to EAs inducing 2-7-fold increase in biofilm formation compared to non-supplemented cultures. Altogether, our work provides the first insights into *S. algae* biofilm physiology, linking two unrelated processes *prima facie* which might have implications in environmental niche partitioning of *S. algae* communities.

PT318 The effect of cultivation media on metabolite profiles of nematicidal Bacillus species

Ilze Horak[1], Peet Jansen van Rensburg[2], Sarina Claassens[1]

[1]*North-West University, Unit for Environmental Sciences and Management, Potchefstroom, South Africa*
[2]*North-West University, Focus Area Human Metabolomics, Potchefstroom, South Africa*

Background: *Bacillus* species are known root-knot nematode antagonists and promising bionematicides. Although various studies attribute nematicidal activity of *Bacillus* species to metabolite production, *in vitro* metabolic activities and how they relate to different cultivation media are not fully understood. The purpose of this study was to investigate the influence complex media versus simpler media on *Bacillus* metabolite profiles through the use of untargeted metabolomics.

Objectives: The objectives were: (i) To determine the best cultivation practices for subsequent metabolomics analysis of nematicidal *Bacillus* species and (ii) to optimise cultivation media for production of nematicidal compounds.

Methods: *Bacillus* species confirmed to have nematicidal activity were cultivated in a complex (Luria-Bertani broth) and a minimal media, after which the resulting bacterial filtrates were analysed by gas and liquid chromatography-mass spectrometry.

Results: In LB broth, more (± 126) practically and statistically significant ($d < 0.8$ and $p > 0.05$) metabolite features were detected, while cultivation in MB yielded fewer (± 20). The latter could be attributed to a simpler broth composition of glucose and salts, as opposed to LB broth containing tryptone and yeast extracts. Consequently, when cultivated in LB broth *Bacillus* have access to more nutrients which can be utilised, whereas in MB it does not. However, both media resulted in the production of compounds that tested positive for nematicidal activity. Further quantitative analyses will determine specific effects of different cultivation media which should be noted as an important consideration in *Bacillus*-based bionematicide development.

PT319 Transcriptional and translational prerequisites for spontaneous DNA uptake in Escherichia coli

Žana Kapustina[1;2], Aistė Liucvaikytė[2], Lukas Taujenis[2], Gytis Mackevičius[2], Arvydas Lubys[1;2]

[1]*Vilnius University, Institute of Biosciences, Life Sciences Center, Vilnius, Lithuania*
[2]*Thermo Fisher Scientific Baltics, Vilnius, Lithuania*

Background: Escherichia coli, which is not usually considered as naturally competent bacteria, was found to spontaneously take up exogenous DNA on agar plates. Analyzing mechanisms underlying this phenomenon is important for understanding the role of transformation in evolution as well as in transfer of pathogenicity or antibiotic resistance genes. Available data suggests that DNA uptake in E. coli is physiologically regulated process, most probably related to the RpoS-dependent response, however global changes in gene expression patterns upon approaching and entering the state of natural competence have not been studied in depth.

Objectives: Here we will present the results of a genome-wide transcriptomic and proteomic profiling study designed to track the laboratory E. coli strain throughout its entrance into the state of natural transformability. We aim to shed light on global mechanisms of natural competence development in E. coli.

Methods: Cells were collected at different time points throughout the natural transformation experiment and used for the extraction of RNA and proteins. Total RNA sequencing revealed global transcriptomic profiles of cells in different growth phases as well as upon plating on agar. Quantitative mass spectrometry was conducted to complement transcriptomic data with global proteomic profiles.

Results: Transcriptional and translational profiles of E. coli cells change significantly upon entering the stationary phase and upon contact with agar. Interestingly, those changes do not depend on the presence of exogenous DNA suggesting that a common non-specific mechanism is capable of mediating DNA uptake.

PT320 The impact of type IV pili and twitching motility in Streptococcus sanguinis

Yi-Ywan Margaret Chen[1], Chia-Hua Wu[1]

[1]*Chang Gung University, Microbiology and Immunology, Taoyuan, Taiwan*

Background: *Streptococcus sanguinis*, a common oral isolate and an opportunistic pathogen, is the only known streptococcus possessing a *pil* gene cluster for the biosynthesis of type IV pili (Tfp). Although this cluster is commonly present on *S. sanguinis* genome, only a few strains express active Tfp-mediated twitching motility on agar plates under anaerobic cultivation. As the activity of PilT ATPase in the twitching active strain is detected microscopically under 5% CO_2 cultivation, it is suggested that the growth atmosphere-regulated twitching activity provides niche-specific advantages for *S. sanguinis*.

Objectives: To determine the impact of Tfp in oral biofilm and niches outside the mouth in twitching active CGMH010 and non-twitching SK36.

Methods: The expression of *pilT* was evaluated by qPCR. The piliation was exmined by transmission elctronmicroscopy. The adherehce to host epithelia and static bifoilm formation in wild-type *S. sanguinis*, its isogenic pilin-null and PilT-null derivatives were analyzed.

Results: *S. sanguinis* CGMH010 expressed a higher amount of *pilT* and produced longer Tfp compared to that of SK36. CGMH010 formed less biofilm than SK36, but inactivation of PilT promoted the biofilm formation of CGMH010. Tfp were essential for optimal adherence of both strains to host epithelia, and PilT activity was required only for the optimal binding of CGMH010. Taken together, it is suggested that the expression of *pilT* and phenotype of Tfp are associated with twitching activity. Active twitching motility may reduce the static biofilm formation but could potentially facilitate the interaction between *S. sanguinis* and host cells.

PT321 The role of imperfect inverted repeats in the target site specificity of the conjugative transposon Tn5397

Sarah Alharbi[1;2], Peter Mullany[2], Adam Roberts[1]

[1]*Liverpool School of Tropical Medicine, United Kingdom*
[2]*UCL Eastman Dental Institute, United Kingdom*

Background: Conjugative transposons (CTns) are discrete segments of DNA that can transfer between bacterial cells and often carry antibiotic resistance genes (ARGs). The multi-drug resistant *Clostridium difficile* strain 630 harbours the antibiotic resistance encoding CTn Tn*5397* which displays considerable target-site specificity upon insertion into the genome. The excision and insertion of Tn*5397* is catalysed by the large serine recombinase, TndX. An important structural feature of the Tn*5397* target-site is the presence of imperfect inverted repeats (IIRs) flanking a central GA dinucleotide.

Objectives: The aim of this study is to investigate the role of IIRs in the target site selectivity of Tn*5397*. This will provide an insight into the molecular mechanism of Tn*5397* insertion preference to be able to direct derivatives of Tn*5397* to insert at specific sites within a genome; for example, within ARGs.

Methods: *In vivo* transposition system (consisting of mini-Tn*5397*, insertion sites in other replicons and TndX *in cis*) was established in *Escherichia coli* to reconstitute the insertion reaction of Tn*5397*. Mutational analysis was performed to convert the IIRs of the target site to perfect inverted repeats. The insertion of the mini-transposons into the target sites was detected by PCR designed to detect the left and the right mini-transposon target junctions of the inserted mini-transposon.

Results: Current data demonstrates successful transposition of the mini-transposon into the wildtype target-site and that converting IIRs into perfect inverted-repeats does not prevent insertion. Therefore, opening the possibility of altering target site specificity and using Tn*5397* as a controllable insertional mutagen.

PT322 OmpR regulatory protein specifically binds at the fur promoter region in Yersinia enterocolitica

Karolina Jaworska[1], Marta Ludwiczak[1], Adrianna Raczkowska[1], Katarzyna Brzostek[1]

[1]*Department of Applied Microbiology, Institute of Microbiology, Faculty of Biology, University of Warsaw, Warsaw, Poland*

Background: OmpR is a transcriptional regulator of two-component transduction system EnvZ/OmpR that controls the expression of genes involved in many different cellular processes and virulence of pathogenic bacteria, including human enteropathogen *Yersinia enterocolitica*.

The concentration of iron in the environment is critical for the control of bacterial metabolism and iron-acquisition systems are considered as important pathogenicity determinants. The global ferric uptake regulator Fur controls iron transport and storage to precise maintenance of iron homeostasis necessary for survival of different pathogenic bacteria. Recently, proteomic analysis revealed the influence of *Y. enterocolitica* OmpR regulator on the production of proteins involved in iron acquisition and storage uncovering so far unknown link between OmpR regulator and iron homeostasis.

Objectives: Bioinformatic analysis revealed the presence of four putative OmpR binding sites within the *fur* promoter region in *Y. enterocolitica.* The aim of this study was to reveal whether OmpR may act as a regulator for *fur* expression by binding at the *fur* promoter region.

Methods: Recombinant OmpR-His6 protein was purified from an overproducing strain. The electrophoretic mobility shift assays and DNase I footprinting were used to study OmpR-DNA interactions.

Results: We demonstrated that the OmpR, specifically recognizes and binds four DNA targets in the *fur* promoter region. Interestingly, OmpR exhibited different affinity for these binding sites. The bandshift assays and DNase footprinting showed that the second OmpR binding site at the *fur* promoter region have highest affinity for OmpR.

This work was supported by grant UMO-2016/21/B/NZ6/01009 from the National Science Centre (Poland).

PT323 Using NMR-based metabolomics to dissect the global consequence of 2-aminoacrylate accumulation in Salmonella enterica

Andrew Borchert[1], Goncalo Gouveia[2], Arthur Edison[3], Diana Downs[4]

[1]*University of Georgia, Dept. of Microbiology, Athens, United States*
[2]*University of Georgia, Dept. of Biochemistry, Athens, United States*
[3]*University of Georgia, Depts. of Biochemistry and Genetics, Institute of Bioinformatics and Complex Carbohydrate Center, Athens, United States*
[1]*University of Georgia, Dept. of Microbiology, Athens, United States*

Background: Metabolic networks are comprised of biochemical reactions organized to maximize growth/replication while minimizing the detrimental impact of reactive metabolites on cell fitness. 2-aminoacrylate (2AA) is an internally generated reactive metabolite that inactivates pyridoxal 5'-phosphate (PLP)-dependent enzymes involved in amino acid metabolism. Some targets for 2AA stress and the physiological consequences are known in *Salmonella enterica*, but the global impact of these perturbations and the ensuing metabolic response of the organism remains relatively unexplored.

Objectives: To determine the effectiveness of using metabolomics to better understand the global metabolic consequence(s) of 2AA accumulation in *S. enterica* and enhance our understanding of metabolic network structure and integration.

Methods: Cell pellet and spent medium samples from isogenic *S. enterica* strains (*ridA+/-*) grown in conditions designed to alter the level of 2AA stress were obtained. The metabolome for each strain under each condition was assessed by ^{1}H-NMR.

Results: Strains lacking *ridA* accumulate 2AA, and as expected, harbor a distinct metabolome from their wild-type parental strains. Metabolomic data supported the conclusion that the dominant physiological perturbations in a *ridA* mutant were due to 2AA, since preventing its formation eliminated the majority of metabolic discrepancies observed. Consistent with past genetic results, a subset of differences was eliminated by glycine addition. This helped define NMR features that can be probed for components of the glycine and one-carbon unit network. Overall, this work outlines an innovative approach to integrate metabolomics with a well-understood physiological stress to identify new metabolic stress outcomes and dissect network structure integration.

PT324 relationship between intestinal microbiota, ghrelin and leptin levels and inflammatory profile of obese end eutrophic women

Anne Brito[1], verena macedo[2], Lucas Miranda Marques[1]

[1]Universidade Federal da Bahia (UFBA) - Campus Anísio Teixeira, Vitória da Conquista, Brazil
[2]University of São Paulo, São Paulo, Brazil

Background: The obesity is a multifactorial disorder characterized by the excess of ectopic body fat with health damage. The intestinal microbiota has been one of the factors associated with obesity and its consequences, due to its physiological interaction with the host.

Objectives: Correlate the nutritional, inflammatory and hormonal profiles to the intestinal microbiota of the obese and eutrophic women.

Methods: 40 women were evaluated. 20 of them were diagnosed with obesity and 20 were in the eutrophic state. We performed the quantification of *Firmicutes* and *Bacteroidetes philos* as well as *Lactobacillus ssp., Clostridium ssp., Bacteroides ssp*. and *Escherichia coli* using PCR real time. In adiction, eighteen cytokines, ghrelin and leptin hormones were dosed in the women serum.

Results: Obese women presented all nutritional variables altered when compared to control. The proportion *Firmicutes/Bacteroidetes* were higher in obese women as well as the quantification of *Lactobacillus ssp*. and *Bacteroides ssp*. However, the quantification of *E.coli* and *Clostridium ssp*. was higher in control group. A positive correlation was observed between IL-6, TNFα, INF-γ and IL-10 and the nutritional variables weight, Body Mass Index (BMI), Total Body Fat and waist-hip ratio. Positive correlation was observed between leptin levels and nutritional variables and negative correlations between ghrelin levels and nutritional variables.

PT325 Multilocus sequence typing analysis of extended-spectrum beta-lactamases producing E. coli isolated from unrelated healthy hosts in the Northern Thailand

Panan Ratthawongjirakul[1], Visanu Thamlikitkul[2], Chakkraphong Seenama[2]

[1]*Chulalongkorn University, Transfusion Medicine and Clinical Microbiology, Bangkok, Thailand*
[2]*Mahidol University, Medicine, Bangkok, Thailand*

Background: Extended-spectrum beta-lactamases producing *E. coli* (ESBL-*E. coli*) has currently disseminated in non-hospitalised people, and domestic and farm animals. These hosts may become a reservoir of a silent spread of antimicrobial resistant bacteria in a community. Therefore, spreading of ESBL-*E. coli* between human and animal has not been thoroughly assessed.

Objectives: This study aimed to analyse a genetic relatedness between ESBL-*E. coli* isolated from the unrelated healthy hosts, a human in the community and swine in the livestock, in Amphor Mueang, Lamphun Province, in the northern of Thailand.

Methods: Two hundred and twelve ESBL-*E. coli* isolated from human and swine were examined for a presence of $bla_{\text{CTX-M}}$, bla_{TEM}, and bla_{TEM} by multiplex-PCR and a whole genome sequencing. The genetic relatedness of the isolates was analysed by multilocus sequence typing.

Results: $bla_{\text{CTX-M}}$ was the most dominant ESBL-encoding gene found in 95.75% of isolates, followed by bla_{TEM} (60.85%) and bla_{TEM} (2.40%). The most common sequence types (STs) identified from human isolates were ST131, ST101, and ST70, and from swine isolates were ST10, ST48, and ST131, respectively. Interestingly, ST131 strains carrying $bla_{\text{CTX-M}}$ were the major isolated ESBL-*E. coli*, supporting the previous study that considered the strain as a truly pathogenic. There were 66.51% of ESBL-*E. coli* that shared identical 19 STs, including a host-restricted ST131, between human and swine suggesting that transmission between these two hosts might be possible. Although, proof for a direct transfer of ESBL-*E. coli* from animal to human, or vice versa, is still required for further elucidation.

PT326 Negative charges within the linker segment of Bordetella adenylate cyclase toxin control enzyme translocation in a calcium dependent manner

Anna Sukova[1;2], Ladislav Bumba[1], Pavel Srb[3;4], Vaclav Veverka[3], Ondrej Stanek[1], Jana Holubova[3], Radovan Fiser[1;2], Peter Sebo[1], Jiri Masin[1]

[1]*Institute of Microbiology of the ASCR, v. v. i., Prague, Czech Republic*
[2]*Faculty of Science, Charles University, Department of Genetics and Microbiology, Prague, Czech Republic*
[3]*Institute of Organic Chemistry and Biochemistry AS CR, Prague, Czech Republic*
[4]*Faculty of Science, Charles University, Department of Cell Biology, Prague, Czech Republic*

Background: The adenylate cyclase toxin (CyaA), secreted by the whooping cough agent *Bordetella pertussis*, exhibits two distinct biological activities that operate simultaneously on target cells. The C-terminal hemolysin (Hly) part forms pores, permeabilizing cell membrane and eventually provoking colloid-osmotic cell lysis. The N-terminal adenylate cyclase (AC) domain of CyaA reaches the cell cytosol and after calmodulin binding subverts phagocyte functions by catalyzing unregulated conversion of cytosolic ATP into cAMP. The AC-to-Hly-linking segment (residues ~ 400 to 500 of CyaA) was proposed to be involved in both activities.

Objectives: The goal of our study was to solve the structure of the AC-to-Hly-linking segment and its biological activities.

Methods: We used nuclear magnetic resonace spectroscopy, fluorescence spectroscopy and black lipid membranes.

Results: We solved the NMR structure of $CyaA_{411-490}$ in the presence of membrane-mimicking dodecylphosphocholine micelles and showed that the segment consists of two α-helices spanning residues Gly418-His439 and His457-Phe485. Our data suggest that the C-terminal helix is in a transmembrane arrangement, whereas the N-terminal helix is in a more solvent-exposed position within the micelles. Purified $CyaA_{411-490}$ polypeptide permeabilized almost exclusively negatively charged liposome vesicles and in contrast to the synthetic peptide SP454-484 it formed stable pores in black lipid membranes of different lipid composition. We further demonstrated that $CyaA_{411-490}$ binds calcium ions with low affinity (Kd ~2.9 mM) and showed, that the clusters of negatively charged residues localized between residues Glu419 to Glu432 and Asp445 to Glu448 appear to control translocation of the AC domain of CyaA in a calcium-dependent manner.

PT327 regulation of the metalloprotease with quorum sensing regulator opar in vibrio parahaemolyticus

San-Chi Chang[1], Chia-Yin Lee[2]

[1]National Taiwan University, Microbiology Laboratory, Agricultural Chemistry, Taipei, Taiwan
[1]National Taiwan University, Microbiology Laboratory, Agricultural Chemistry, Taipei, Taiwan

Background: *Vibrio parahaemolyticus* is a Gram-negative bacterium which inhabits the marine and estuarine environments. It is also a human pathogen and causes food-borne gastroenteritis. Quorum sensing (QS) is a signal transduction system for bacteria to communicate with each other. Bacteria change their behavior by detecting the concentration of extracellular chemicals, autoinducers. The gene expression in a microbial community is synchronised by QS.

Objectives: Numerous studies have shown that virulence proteases are associated with the QS system in *Vibrio* spp. Therefore, we investigated the correlation of QS and metalloprotease genes in this study.

Methods: ChIP-seq was used for searching the OpaR-regulated protease genes in the *Vibrio parahaemolyticus* genome. Then, qPCR and luxAB assay demonstrated the regulation among regulator, OpaR and targeted protease genes. The directly binding sequences of OpaR on targeted protease genes are confirmed using EMSA and Footprinting.

Results: We found three metalloprotease genes, LytM (VPA1649), Mcp02 (VPA0755) and M6 protease (VP0907) directly regulated by OpaR at both late-log and stationary phases. The gene expression of Mcp02 and M6 protease increased 6-fold and 4-fold at late-log phase comparing to log phase. At stationary phase, LytM showed 4-fold increasement in contrast with the gene expression at log phase. OpaR repressed the gene expression of them and bound to their promoter regions. The OpaR binding sequences are located on -529 to -510 for LytM, -323 to -303 for Mcp02 and -340 to -320 for M6 protease from their translation start sites.

PT328 A new twist in the regulatory landscape of Escherichia coli

Isabella Moll[1]

[1]*Max F. Perutz Laboratories, University of Vienna, Department of Microbiology, Immunobiology and Genetics, Vienna,*

Background: In their natural environment, Bacteria encounter a variety of stressors. Thus, they are equipped with diverse mechanisms to adapt to these dynamic environmental cues. Hitherto, the alteration of gene expression in response to stress was mainly attributed to alternative transcription or the regulation of protein synthesis by means of protein or RNA factors that affect translation initiation.

Objectives: We focus on the modulation of the translatome *via* reversible ribosome heterogeneity that allows bacteria to achieve a fast and energy-efficient stress response.

Results: Here, we will provide evidence that this mechanism has the potential to reshape established regulatory pathways in *Escherichia coli*, thus emphasizing the key role of the translational machinery in the economic integration of environmental signals in the stress response network.

PT329 Bacteriocin-induced mutations activate antibiotic resistance modules in Lactococcus lactis

María Jesús López-González[1], Ana Belén Campelo[1], Susana Escobedo[1], Ana Rodríguez[1], Ana Rute Neves[2], Thomas Janzen[2], Beatriz Martinez[1]

[1]*Instituto de Productos Lácteos de Asturias IPLA - CSIC, Villaviciosa, Spain*
[2]*Chr. Hansen A/S, Hørsholm, Denmark*

Background: Adaptive evolution experiments under cell envelope stress exerted by the lipid II-binding bacteriocin Lcn972 selected for evolved Lcn972R *L. lactis* mutants resistant to bacitracin and nisin. Genome sequencing identified mutations in the *ysaDCB* operon coding for a putative bacitracin ABC-transporter (BceAB-like) involved in sensing and resistance to antimicrobial peptides.

Objectives: Our goal was to assess the role of the mutations found in the YsaB permease.

Methods: The genes and their mutated versions were cloned and expressed on a defective *ysaB L. lactis*. Their contribution to resistance was determined by IC50 assays and their ability to sense bacitracin and activate transcription was quantified by promoter fusions.

Results: Expression of the wild type genes resulted in a 4-fold increase in resistance to bacitracin, demonstrating their function as a bona-fide BceAB-like ABC transporter in *L. lactis*. Mutated versions, however, were more effective increasing up to 9- and 5-fold bacitracin IC50 values. Moreover, counter mutations could be selected that restored the antibiotic sensitive phenotype. Promoter fusions revealed that mutated versions had an increased signalling activity and, therefore, a higher basal expression of the *ysaDCB* operon in the absence of bacitracin, i.e. without inducer. To sum up, bacteriocin pressure may select for constitutively expressed and functionally enhanced efflux pumps that interfere with antibiotic activity.

PT330 Developing protein antibiotics for AMR Pseudomonas aeruginosa

Madhuri Barge[1], Anne Six[1], Daniel Walker[1]

[1]*Infection, Immunity and Inflammation, MVLS, Glasgow, United Kingdom*

Background: Antibiotic resistance is a global health threat and without discovery of new antibiotics we are heading towards a pre-antibiotics era where bacterial infections will become deadly. In our lab we work on a group of protein antibiotics (bacteriocins) produced by gram negative bacteria that have evolved to win and survive the intra species competition. One such protein antibiotics is pyocin S5, produced by *Pseudomonas aeruginosa*.

Objectives: We intend to develop pyocin S5 as a highly specific therapeutic for the treatment of *Pseudomonas aeruginosa*, the major cause of death in patients suffering from cystic fibrosis. The primary objective is to generate methods for the production, formulation, manufacture and delivery of pyocin S5 and a safety profile for delivery by inhalation.

Methods: To achieve this we have developed pyocin S5 purification method. We have lyophilised pyocin S5 in various solutions and tested the lyophilised protein at various time interval and temperatures to evaluate suitable formulation conditions. The long term and short-term stability tests were carried out for the formulated protein.

Results: Pyocin S5 has a great potential for development as a much needed therapeutic drug to treat antibiotic resistant *Pseudomonas aeruginosa* infection and might be a suitable drug for treating people with cystic fibrosis.

PT331 Persistence as a microbial survival strategy against disinfectants

Niclas Nordholt[1], Dominique Lewerenz[1], Selina Schmidt[1], Frank Schreiber[1]

[1]*Federal Institute for Materials Research and Testing (BAM), Department of Materials and Environment, Berlin, Germany*

Background: One cornerstone to prevent the spread of antibiotic resistant bacteria in clinical settings is the application of disinfectants. However, bacteria can evolve resistance to disinfectants, which in turn can confer cross-resistance to antibiotics. Additionally, clonal bacterial populations can display phenotypic heterogeneity with respect to the tolerance of antibiotic stress leading to a prolonged survival of a sub-population; this phenomenon is termed persistence. Persistence to antibiotics is an evolvable trait and can serve as a stepping stone for the evolution of genetically encoded resistance. Until now, there is a lack of systematic studies that investigate if bacterial populations establish persister subpopulations that tolerate disinfectant stress longer than most of the population.

Objectives: Our objective is to investigate if persistence is a bacterial survival strategy against disinfectants. Furthermore, we investigate the mechanisms of disinfectant persistence and if persistence can evolve in the face of fluctuating exposure to disinfectants. Lastly, we test if the evolved mechanisms of disinfectant tolerance lead to disinfectant resistance and if they confer cross-tolerance and cross-resistance against antibiotics.

Methods: We use time-kill assays in the presence of disinfectants and experimental evolution combined to whole-genome sequencing in the model organism *E. coli*.

Results: We find persister sub-populations against chlorhexidine and quaternary ammonium compounds, but not to alcohols, aldehydes and oxidative compounds. We will present the relationship of mechanisms known to underlie antibiotic persister formation to the formation of persisters against disinfectants. In addition, we will present data from an ongoing evolution experiment for persistence against disinfectants.

PT332 Short term evolution experiments and comparative genotyping and phenotyping of ancestors and evolved strains adds to understanding L. monocytogenes survival strategies

Tjakko Abee[1], Jeroen Koomen[1], Natalia Crespo[1], marcel zwietering[1], heidy den Besten[1]

[1]*Wageningen University, Laboratory of Food Microbiology, wageningen,*

Background: *Listeria monocytogenes* is a foodborne pathogen notorious for its robustness, and further insight is required in mechanisms underlying adaptive stress response and survival strategies.

Objectives: To determine stress-induced mutation rates in *L. monocytogenes* and to use selected strains in experimental evolution studies followed by genotyping and phenotyping of ancestors and evolved variants obtained after exposure to food and host relevant stresses.

Methods: Mutation rates were determined using a high throughput Luria-Delbrück protocol and selection for *rpoB*-conferred rifampicin resistance, and genotyping and phenotyping of ancestors and evolved variants.

Results: Mutation rates of 20 isolates, including *L. monocytogenes* EGDe, varied from 7.6^{-10} to 6.7^{-9} mutations per cell per generation, while one food isolate, strain FBR16 with a mutation in DNA mismatch repair gene *mutS*, generated 7.8^{-8} mutations per cell per generation. Studies revealed that exposure to food-relevant stresses did not significantly increase mutation rates. *L. monocytogenes* EGDe and FBR16, and LO28 multiple stress resistant variants with reduced fitness were used in evolution experiments to select for isolates with enhanced biofilm formation capacity and fitness, respectively. Evolved EGDe and FBR16 variants showing significantly higher biofilm formation capacity were isolated and comparative genome sequence analysis and phenotyping of ancestor and evolved variants revealed specific mutations in genes linked to a range of functions including stress response, antibiotic resistance and cell wall modifications. Evolved L28 strains with enhanced fitness and altered stress resistance showed mutations in genes encoding 30S ribosomal proteins. Results obtained provide novel insight on *L. monocytogenes* stress adaptation and survival strategies.

PT333 The alterations in growth of UPEC in the presence of human hormones: A pilot study in cell culture

Mine Anğ Küçüker[1], Gül İpek Gündoğan[2], Fatma Kal ayci Yüksek[1], Defne Gümüş[1]

[1]*İstanbul Yeni Yüzyıl Üniversitesi, Medical Faculty, Medical Microbiology, Turkey*
[2]*İstanbul Yeni Yüzyıl Üniversitesi, Histology and Embriology, Turkey*

Background: When an infection occurs, bacteria must withstand stress conditions induced by diverse environmental factors in their host, which affect microbial survival.

Objectives: It was aim to analyze the effects of human insulin (Ins), norepinephrine (NE) and estradiol (Est) on growth of a uropathogenic E. coli strain (UPEC-C7) in an infection model in Human lung cell A549.

Methods: Cell culture was prepared in 96-well plates with 10% FBS added Dulbecco's Modified Eagle's Medium (DMEM). 107 CFU/mL overnight culture of UPEC was also prepared. 20 and 200 µU insulin; 0.0017 and 0.04µg/mL norepinephrine; 0.1 and 0.4 ng/mL estradiol were used according to their physiological levels. Growth was determined via optical density measurement in a spectrophotometer followed by three hours of incubation. Statistical analyses were done by using one sample t test and one-way ANOVA followed by Tukey's multiple-comparisons test.

Results: The growth of UPEC has been decreased significantly in the presence of high level Est and high level NE ($p< 0.05$). Cell line significantly enhanced the growth of UPEC ($p< 0.0001$). Besides, the growth of UPEC was significantly enhanced with the coexistence of host cell and high Ins; but the coexistence of host cell and low Est was found to decrease the growth of UPEC significantly ($p< 0.05$). It has been suggested that, mammalian hormones affect the pathogenicity of infectious diseases as an environmental factor because they are known to change microorganisms' behaviors. It was also shown that, hormones may act differently on growth of bacterium depend on culture conditions.

PT334 Characterization of transcriptional regulator PA3458 from Pseudomonas aeruginosa

Karolina Kotecka[1], Adam Kawałek[1], Aneta Agnieszka Bartosik[1]

[1]*Institute of Biochemistry and Biophysics Polish Academy of Sciences, Department of Microbial Biochemistry, Warsaw, Poland*

Background: *Pseudomonas aeruginosa* is a ubiquitous Gram-negative bacterium and a common cause of life-threatening nosocomial infections. Its exceptional adaptability involves complex regulatory systems, engaging approximately 500 known and potential transcriptional regulators (TRs), constituting almost 10% of all encoded proteins. Among them, 11 are classified as a MarR-type TRs. Most of the so far characterised MarR regulators act as transcriptional repressors which are involved in the control of processes vital for bacterial pathogens, including adhesion, virulence and antibiotic resistance.

Objectives: The aim of this work was a functional characterization of the MarR-type regulator PA3458 from *P*. *aeruginosa*.

Methods: *P*. *aeruginosa* PAO1161 strain overproducing PA3458 was obtained by introducing the expression vector with *pa3458* gene under the control of inducible *araBAD*p promoter. RNA-seq, qPCR and electrophoretic mobility shift (EMSA) assays, using purified His-tagged protein, were applied in functional characteristics of PA3458.

Results: Overproduction of PA3458 had a negative impact on the growth of *P*. *aeruginosa.* Transcriptional profiling of PAO1161 strain with a mild PA3458 excess revealed 74 down- and only 22 up-regulated genes, suggesting that PA3458 acts as a repressor. Sixteen of the down-regulated genes encoded proteins involved in amino acids metabolism. These included *pa3459-pa3461*operon, oriented divergently to *pa3458*, with a putative role in glutamate metabolism. Complementary qPCR analysis of *pa3458* mutant showed significant up-regulation of the operon. Together our data suggests that PA3458 acts as the transcriptional regulator controlling amino acid metabolism in *P. aeruginosa*.

This work was supported by the National Science Centre in Poland (grant 2015/18/E/NZ2/00675).

PT335 Identification and characterization of ROS- independent tellurite targets in Escherichia coli

Roberto Luraschi[1], Fabián Cornejo[1], Constanza Castro[1], Claudia Muñoz-Villagrán[1], Felipe Arenas[1], Jeff S. Piotrowski[2], Eduardo H. Morales[3], Claudio C. Vásquez[1]

[1]*Universidad de Santiago de Chile, Facultad de Química y Biología, Santiago, Chile*
[2]*Yumanity Therapeutics, Massachusetts, United States*
[3]*uBiome Inc., San Francisco, United States*

Background: The Te oxyanion tellurite is highly toxic to bacteria. To date, most studies aimed at elucidating the mechanisms of toxicity and intracellular tellurite targets in aerobic conditions. Once inside the cell, tellurite oxidizes sulfhydryl groups of proteins and results in the generation of superoxide and other reactive oxygen species (ROS). Thus, it has been argued that ROS are the main responsible for tellurite toxicity. However, recent results indicated that there is no correlation between exposure to tellurite and hydrogen peroxide or superoxide resistance, suggesting that tellurite is toxic *per se*.

Objectives: Identify molecules, genes, metabolic pathways, or so, affected by tellurite in the absence of ROS.

Methods: All experiments were conducted using *E. coli* BW25113 grown in minimal M9 medium under aero- or anaerobiosis. Genes involved in tellurite resistance were identified by chemical genomics (CG) profiling using a pool of 6,000 *E. coli* deletion mutants coupled with high throughput sequencing. Differences between metabolite concentration in control and tellurite-exposed *E. coli* were determined by a metabolomic approach.

Results: Tellurite anaerobic minimum inhibitory concentration for *E. coli* increased 100-fold as compared to aerobiosis. CG profiling showed that tellurite affected several metabolic pathways including the Krebs cycle, phospholipid synthesis, and synthesis and degradation of amino acids, ribo- and deoxyribonucleotides. This suggests that tellurite-mediated damage hampers *E. coli* to repair and survive the stress imposed by this oxyanion.

PT336 The role of cell-cell communication in Bacillus subtilis floating biofilms

Mihael Spacapan[1], Tjaša Danevčič[1], Polonca Stefanic[1], Ines Mandic-Mulec[1]

[1]*University of Ljubljana, Biotechnical faculty, Department of Food Science and Technology, Chair of Microbiology, Ljubljana, Slovenia*

Background: *Bacillus subtilis*, is a Gram-positive spore forming and industrially important bacterium, which forms floating biofilms (pellicles) in liquid cultures. The ComQXPA cell-cell communication system, also known as the quorum sensing system, up-regulates a diverse array of genes (e.g. exoprotease, biofilm matrix component and sporulation genes). How the extracellular signaling peptide ComX increases the quantity of the end products encoded in the above mentioned genes in pellicles is still unknown.

Objectives: Our aim was to determine the role of ComX in pellicles. We hypothesized that ComX increases the quantity of exoproteases, matrix components and spores in pellicles.

Methods: A *B. subtilis* signal deficient *comQ* mutant pellicle was compared to the wt (wild type) pellicle. Promoter activity in pellicles carrying fluorescent reporter fusions was estimated with a confocal microscope and a plate reader. Proteolytic activity was determined by casein gelatin assays. Pellicle matrix components (sugars, proteins) were isolated and quantified by biochemical assays. ComX biological activity and proteolytic decay was determined with a biosensor assay and high performance liquid chromatography, respectively.

Results: We found that ComX increases exoprotease quantity but does NOT increase pellicle formation and does NOT significantly increase spore counts after 40 h of incubation. Nevertheless, ComX dramatically changes the dynamics of sporulation and growth, which could explain why the pellicle formation of the signal deficient mutant is not decreased, as was expected. Additionally, ComX is susceptible to loss of biological activity in highly proteolytic environments.

PT337 The CydX-dependent cytochrome bd oxidase in Salmonella Typhimurium

Duc Kieu Minh[1], Iel Soo Bang[1]

[1]*Chosun University, Department of Microbiology and Immunology School of Dentistry, Gwangju, Korea, Rep. of South*

Background: Most bacteria possess *bo*- and *bd*-type oxidases for terminal cytochrome oxidases that transfer electrons in their hemes to molecular oxygen in electron transport chain. Because the cytochrome *bd* oxidase has higher affinity to oxygen than the heme-copper *bo* oxidase, it has been revealed critical for the replication and virulence of facultative anaerobic pathogens invading less oxic host tissues. Recent studies in some bacteria have identified a new small protein CydX for the activity of the cytochrome *bd* oxidase complex, in addition to CydA and CydB subunits, proposing it as a new antimicrobial target.

Objectives: We investigated if the CydX homologue in *Salmonella* Typhimurium was required for consisting of the cytochrome *bd* complex and for the cytochrome *bd* oxidase-dependent metabolism in *Salmonella.*

Methods: Phenotypes of mutant *S.* Typhimurium lacking CydX were examined in the assembly and activity of the cytochrome *bd* oxidase, and the resistance against antimicrobials including β-mercaptoethanol, nitric oxide, and antibiotics, and against antimicrobial activities in macrophages.

Results: Mutant *S.* Typhimurium lacking CydX showed loss of heme in cytochrome *bd*, impaired oxidase activity for bacterial respiration, comparable to those of *cydAB* mutant lacking both CydA and CydB subunits. Moreover, *cydX* mutants showed increased susceptibility to β-mercaptoethanol and nitric oxide, and the impaired survival inside macrophages. However, *cydX* and *cydAB* mutants showed a dramatic resistance against aminoglycosides including kanamycin and neomycin, suggesting that targeting CydX as a new antimicrobial strategy is promising, but should be considered the potential drug tolerance response induced by inhibiting the *bd* oxidase activity.

PT339 Protein aggregation in Escherichia coli by soft-metal(loid)s during anaerobiosis

Fabian Cornejo[1], María Sandoval-Díaz[1], Roberto Luraschi[1], Claudia Muñoz-Villagrán[1], Camila Cancino[1], Eduardo Morales[2], Jeff Piotrowski[3], Felipe Arenas[1], Claudio Vásquez[1]

[1]*Universidad de Santiago de Chile, Biology, Santiago, Chile*
[2]*uBiome Inc., San Francisco, United States*
[3]*Yumanity Therapeutics*

Background: Non-essential metal(loid)s such as Hg, Au, Ag, As, Te and Cd, among others, are extremely toxic even at low concentrations and do not exhibit a biological role to date. Interestingly, an important number of these metal(loid)s can be classified as soft-metal(loid)s because they share some chemical properties as thiol reactivity and high polarizability. In bacteria, soft-metal(loid)s provoke mainly ROS-mediated cellular damage. Direct molecular targets of these metal(loid)s are almost unknown with the exception of iron-sulfur dismantling.

Objectives: Given the similarity of their chemical properties, the aim of this work is to understand what the targets of these metal(loid)s in ROS-free conditions (anaerobiosis) are.

Methods: During a chemical genomics analysis, the entire pool of mutants was independently exposed to the soft-metal(loid)s Ag, Au, As, Cd, Hg and Te. The abundance of each mutant was quantified by Illumina sequencing and the Chemical Genomic score(CG) was calculated. Enrichment analysis was carried out to find out common cellular targets and common cell responses to these metal(loid)s. Protein aggregation was determined and aggregates were identified and quantified by LFQ proteomics (Bioproximity)

Results: Since mutants in response to protein misfolding were significantly depleted upon soft-metal(loid)s treatment, we hypothesize that soft-metal(loid)s provoke ROS-independent protein aggregation in *E. coli*. Aggregation includes mainly Fe-S cluster-containing proteins and enzymes involved in amino acid biosynthesis. Given that aggregation still occurs in translation-blocked cells, suggesting that the metal(loid)s target already synthetized proteins.

PT340 The Peculiarities of Phe-Arg-beta-naphthylamide (PABN) and 1-(1 Naphthylmethyl)piperazine (NMP) interaction with Salmonella enterica and Listeria monocytogenes cells

Sandra Sakalauskaitė[1], Rimantas Daugelavičius[2]

[1]*Vytautas Magnus university, Department of Biochemistry, Kaunas, Lithuania*
[2]*Vytautas Magnus University, Department of Biochemistry, Kaunas, Lithuania*

Background: Antimicrobial resistance is a steadily growing worldwide problem. Efflux pumps play a key role in resistance to antibiotics. It is not easy to develop new antimicrobial compounds which would not be efflux substrates. Also, unknown sides effects of new antimicrobials slow down their introduction into medical practice. So, the knowledge of ways to increase the efficiency of well-known antimicrobial compounds would be very useful. Beside this, it is very important to know ways of regulation of efflux in bacteria.

Objectives: The aim of our studies: explore effects of two efflux inhibitors - Phe-Arg-β-naphthylamide (PAβN) and 1-(1 Naphthylmethyl)piperazine (NMP) - on accumulation of indicatory ions in bacteria. Also, ability of these inhibitors to increase the activity of Chloramphenicol, Tetracycline and Ampicillin was studied. We used wild-type cells and efflux pump mutants to determine, if the mutations in pump genes affect the efficiency of inhibitors and the sensitivity of cells to antibiotics. In addition, effects of these Gram-negative bacteria efflux inhibitors were analysed using Gram-positive cells.

Methods: We used tetraphenylphosphonium and ethidium ions, these lipophilic cations selective electrodes and fluorescence measurements to assay efflux activity in *Salmonella enterica* and *Listeria monocytogenes* cells.

Results: We determined that PAβN and NMP enhance accumulation of efflux pumps substrates, increase activity of Chloramphenicol, but not Tetracycline, on wild-type and mutant *S. enterica* cells and have an outer membrane-permeabilizing activities. *L. monocytogenes* bacteria accumulate large amount of this PAβN. Beside this, in *L. monocytogenes* cells PAβN and NMP inhibited efflux of tetraphenylphosphonium, but not ethidium ions.

PT341 Alternative fate of glyoxylate during acetate and hexadecane metabolism in Acinetobacter oleivorans DR1

Chulwoo Park[1], Bora Shin[1], Woojun Park[1]

[1]Korea University, Department of Environmental Science and Ecological Engineering, Seoul, Korea, Rep. of South

Background: The glyoxylate shunt (GS), involving isocitrate lyase (encoded by *aceA*) and malate synthase G (encoded by *glcB*), is known to play important roles under several stress conditions or certain carbon source metabolism (acetate and hexadecane).

Objectives: We focused on analyzing unpredicted growth of GS-deficient DR1 cells under 0.1% acetate and hexadecane.

Methods: All growth tests for *Acinetobacter oleivorans* DR1 were conducted at 30°C in minimal salt basal media. The expression level of *aceA* and *glcB* was examined by Northern blot. Transcriptomes of WT and *aceA*-null mutant were analyzed based on RNA-seq. Analysis of organic acids in DR1 strain was perfomed using high-performance liquid chromatography.

Results: Comparative growth analyses revealed that *aceA*, but not *glcB*, is essential for cells to grow on either acetate (1%) or hexadecane (1 %) in *A. oleivorans* DR1. Interestingly, the *aceA* knockout strain was able to grow slower in 0.1 % acetate than the parent strain. The expression of *aceA* was dependent on the concentration of acetate or H_2O_2, while *glcB* was constitutively expressed. Up-regulation of stress response-related genes and down-regulation of main carbon metabolism-participating genes in a Δ*aceA* mutant suggested that an *aceA* mutant is susceptible to acetate toxicity, but grows slowly in 0.1% acetate. However, a Δ*glcB* mutant showed no growth defect in acetate or hexadecane and no susceptibility to H_2O_2, suggesting the presence of an alternative pathway to eliminate glyoxylate toxicity. Oxalate production and impaired growth of a Δ*ldh*Δ*glcB* double mutant suggested that lactate dehydrogenase is a potential detoxifying enzyme for glyoxylate.

PT342 Extracellular polysaccharides of the phytopathogenic bacterium Pectobacterium atrosepticum: identification, structure, functions.

Bakhtiyar Islamov[1], Vladimir Gorshkov[1;2], Olga Petrova[1], Polina Mikshina[1], Vladimir Vorob'ev[1;2], Marina Ageeva[1], Genadi Burygin[3], Yuri Gogolev[1;2]

[1]Kazan Institute of Biochemistry and Biophysics, FRC Kazan Scientific Center, Russian Academy of Sciences, Kazan, Russian Federation
[2]Kazan Federal University, Kazan, Russian Federation
[3]Institute of Biochemistry and Physiology of Plants and Microorganisms, Russian Academy of Sciences, Saratov, Russian Federation

Background: Bacterial extracellular polysaccharides (EPS) are structurally diverce polymers well-know to determine many bacterial properties (adhesion, resistance, virulence, biofilm formation, etc). Plant soft rot-causing phytopathogen Pectobacterium atrosepticum (Pba) is able to form various 3D biofilm-like structures (including bacterial emboli); however, an extracellular matrix and EPS were not described for this bacterium.

Objectives: We were aimed to identify Pba EPS, determine their structure, assess if they are synthesized in planta, and reveal their potential roles in Pba-host interactions.

Methods: EPS were extracted and then purified and analyzed by size-exclusion and anion-exchange chromatography. To establish EPS structure, one-dimensional (1H, 13C, 13C JMOD), two-dimensional heteronuclear (HSQC, HMBC) and homonuclear (COSY, TOCSY, ROESY) NMR-specta were analyzed. EPS were detected in planta by immunocytochemistry. Physical properties were assesed by dynamic light scattering and viscosimetry. Elicitor properties were examined using several test-systems.

Results: EPS backbone consisted of repeating trisaccharide [→3)-α-D-Galp-(1→2)-α-D-Manp-(1→4)-α-L-Rhap-(1→], the side chain of β-D-Erwiniose-(1→3)-α-D-Galp-(1→ was attached to mannopyranosyl residue. EPS were shown to be synthesized in the host-plant during the formation of bacteria emboli. Two hypotheses on the functional role of EPS in the infection were checked. Firstly, EPS were able to form big aggregates (450 nm) and increase rheological properties of liquids, thus facilitating the colonization of xylem vessels. Secondly, EPS induced the increase in callose deposition (but not ROS accumulation or programmed cell death) in plants, indicating that EPS acted as weak elicitors. The study is supported by RSF (No 19-14-00194).

PT343 High-efficiency secreted expression in Bacillus subtilis of a thermo-alkaline mannanase from an alkaliphilic Bacillus clausii

Yanfen Xue[1], Cheng Zhou[1], Yanhe Ma[1]

[1]*Institute of Microbiology, State Key Laboratory of Microbial Resources, Beijing, China*

Background: β-mannanase catalyzes randomly the cleavage of β-1,4-linked linkages of mannan backbone. Its potential uses have been shown in biobleaching of pulp and paper, detergent additive, hydraulic fracturing of crude oil recovery, and so on. Alkaline and thermostable β-mannanases provide obvious advantages for such applications. However, only a few of them are commercially exploited as wild or recombinant enzymes, and few heterologous and secretory expression of alkaline β-mannanase in *Bacillus subtilis* expression system was reported.

Objectives: The high-level secretory expression in *B. subtilis* was constructed and the β-mannanase (BcManA) from *Bacillus clausii* was expressed in *E. coli* and *B. subtilis*.

Methods: The β-mannanase gene was cloned by constructing gene library and expressed by PCR. The six signal peptides and two strong promoters were tested for efficiently secreted expression optimization in *B. subtilis*.

Results: BcManA exhibited maximum activity at pH 9.5 and 75 °C with good stability at pH 7.0–11.5 and below 80°C. It had high cleavage capability on polysaccharides containing β-1,4-mannosidic linkages, such as konjac glucomannan. The highest specific activity of 2366.2 U mg^{-1} was observed on konjac glucomannan with the Km and kcat value of 0.62 g l^{-1} and 1238.9 s^{-1}, respectively. The highest extracellular activity of 2374 U ml^{-1} with secretory rate of 98.5% was obtained using SPlipA and P43 after 72 h cultivation in 2 × SR medium. By medium optimization using cheap nitrogen and carbon source of peanut meal and glucose, the extracellular activity reached 6041 U ml^{-1} after 72 h cultivation.

PT344 DNA binding properties and localization of paralogous SSB proteins from Streptomyces coelicolor

Tina Paradžik[1], Vlatka Godinić Mikulčić[1], Anja Kostelac[2], Dušica Vujaklija[1]

[1]Ruđer Bošković Institute, Department for physical chemistry, Zagreb, Croatia
[2]University of Zagreb, Faculty of science, Zagreb, Croatia

Background: Streptomycetes are renowned for their capacity to produce diverse range of pharmaceutically important compounds. Two-thirds of clinically relevant antibiotics are produced by streptomycetes. Beside, these bacteria exhibit a complex and tightly regulated developmental life programme. Single-strand DNA-binding (SSB) proteins have a key role in DNA replication, repair and recombination in all domains of life.

SSB act as recruitment platform for genome maintenance proteins and modulates their activities. Many bacteria have additional copies of *ssb* genes, but their biological role is poorly understood. We found that paralogous *Strepomyces coelicolor* SsbA is essential while SsbB participate in chromosomal segregation during sporulation. Their affinity for ssDNA was not examined in detail until now.

Objectives: To analyze in detail interaction of SsbA and SsbB with single-stranded DNA and to examine localization of these paralogous proteins during *S. coelicolor* growth.

Methods: Genes encoding for His-tagged SsbA and SsbB proteins were cloned into high number copy plasmids. SSBs were expressed in *E. coli* and purified using affinity and ion-exchange chromatography. EMSA and fluorescence spectroscopy were used to analyze SSBs affinity to ssDNA. Various genetic constructs encoding for a fused GFP or mCherry SSBs were prepared to analyse localization of these proteins in hyphae during bacterial growth.

Results: The shortest oligonucleotide bound by SsbA and SsbB was determined under different binding conditions. SsbA:ssDNA was found to be much more dependent on salt concetration compared to SsbB:ssDNA. Localization and occasional co-localization of fluorescently labelled SSB proteins were observed in the vegetative and aerial hyphae of *S. coelicolor.*

PT345 High ofloxacin concentrations increase persister fraction in E.coli

Tatjana Schlechtweg[1], Laurence Van Melderen[1], Frédéric Goormaghtigh[1]

[1]*Université libre de Bruxelles, CMM, Gosselies, Belgium*

Background: Together with the current multidrug resistance crisis, bacterial persistence plays an increasingly important role in the failure of antibiotic treatments. Persisters are rare phenotypic variants that acquired transient tolerance to lethal doses of antibiotics. Due to their transiency and low frequency (~1/10,000) the physiology of persister cells is particularly difficult to characterize and so far, not much is known about the molecular mechanisms.

Objectives: Our group is interested in how persister cells are able to elude bactericidal action of ofloxacin, a fluoroquinolone targeting DNA-gyrase, inhibiting replication and inducing the SOS response. Previous works showed that fluoroquinolone persisters rely on a functional SOS response, as also seen in the largely decreased rate of persisters in SOS-deficient cells (LexA3 mutant).

Methods: We investigated the impact of increasing ofloxacin concentrations on survival in correlation with the induction of SOS in exponentially growing *E. coli* cultures. A fluorescent transcriptional reporter (p*sulA::gfp*) coupling the expression of GFP to a SOS promoter was used to monitor the induction of the SOS response.

Results: We found that exposure to extreme concentrations of ofloxacin gave rise to more persister cells than intermediate concentrations. Importantly, the survival rates correlated with the level of SOS induction, indicating a direct link between SOS and survival. Molecular mechanisms underlying this phenomenon are currently being studied.

PT346 A pgpg-specific phosphodiesterase in vibrio cholerae

Jaewoo Lee[1], Yongdae Jang[2], Nam-Chul Ha[2], Yeong-Jae Seok[1;3]

[1]Seoul National University, Department of Biophysics and Chemical Biology, Seoul, Korea, Rep. of South
[2]Seoul National University, Department of Agricultural Biotechnology, Research Institute for Agriculture and Life Sciences, Center for Food Safety and Toxicology, Seoul, Korea, Rep. of South
[3]Seoul National University, School of Biological Sciences and Institute of Microbiology, Seoul, Korea, Rep. of South

Background: The bacterial second messenger cyclic diguanylate monophosphate (c-di-GMP) controls various cellular processes in bacteria. C-di-GMP is enzymatically synthesized by GGDEF domain-containing diguanylate cyclases and degraded by HD-GYP domain-containing phosphodiesterases (PDEs) to 2GMP or by EAL domain-containing PDE-As to 5'-phosphoguanylyl-(3',5')-guanosine (pGpG).

Objectives: Since excess pGpG feedback inhibits PDE-A activity and thereby can lead to uncontrolled accumulation of c-di-GMP, a PDE(s) degrading pGpG to 2GMP (PDE-B) has been assumed to exist. Recently, it has been shown that the oligoribonuclease Orn harbors PDE-B activity in *Pseudomonas aeruginosa*, although it exhibits a broad substrate specificity toward di- to pentaribonucleotides (nanoRNAs) in addition to pGpG.

Methods: Biochemical assay, protein crystallization, structural analysis.

Results: Here, we identified a pGpG-specific PDE-B that we named PggH in *Vibrio cholerae*. While PggH possesses a DHH/DHHA1 domain which is usually responsible for the exonuclease activity toward various substrates, it could not degrade pApA and other nanoRNAs. High-resolution structure of PggH reveals the basis for its narrow substrate specificity. The unique dimeric assembly of the enzyme appears to reduce the structural flexibility of the active site, and thereby allow the access of only pGpG. The substrate specificity and the lower Km of PggH than that of Orn for pGpG indicate that PggH could be the major PDE-B enzyme in *V. cholerae*. Increased expression of PggH during stationary phase and higher sensitivity of the pggH mutant to oxidative stress suggest that the PggH activity might be implicated in completion of the c-di-GMP signaling pathway under stress conditions in *V. cholerae*.

PT347 Mycovirus-induced alteration of stress enzymes' activity and epigenetic changes in phytopathogenic fungus Cryphonectria parasitica

Lucija Nuskern[1], Mirta Tkalec[1], Marin Jezic[1], Zlatko Liber[1], Jelena Mlinarec[1], Zorana Katanic[2], Ljiljana Krstin[2], Mirna Curkovic-Perica[1]

[1]*University of Zagreb, Faculty of Science, Department of Biology, Zagreb, Croatia*
[2]*University of J.J. Strossmayer in Osijek, Department of Biology, Osijek, Croatia*

Background: Chestnut blight fungus *Cryphonectria parasitica* is a pathogenic ascomycete introduced from Eastern Asia to North America and Europe, where it causes the aforementioned disease, devastating to chestnut trees. The disease can be biologically controlled utilising *Cryphonectria hypovirus 1* (CHV1), a mycovirus which changes the physiology of the host, reducing its virulence towards chestnut.

Objectives: The objective of the study was to determine whether the decrease of fungal virulence is caused by epigenetic changes and/or stress induction in the fungus after CHV1 infection.

Methods: Six CHV1 strains of different subtypes were transferred to three *C. parasitica* isolates in order to assess the effect of different CHV1 strains and/or subtypes on global cytosine methylation level in infected fungus, using methylation-sensitive amplification polymorphism (MSAP). Furthermore, the activity of stress enzymes catalase, glutathione S-transferase and superoxide dismutase was measured in CHV1-infected and uninfected fungal isolates.

Results: Infection with CHV1 affected fungal stress enzymes' activity and the methylation pattern of *C. parasitica* genome. The increase in methylation levels correlated well with the CHV1-induced reduction of fungal growth *in vitro*, indicating that *C. parasitica* genome methylation upon CHV1 infection, rather than being the defensive mechanism of the fungus, is more likely to be the virulence determinant of the virus. Furthermore, the severity of CHV1 effect on stress enzymes' activity and methylation levels of infected *C. parasitica* isolates depended mostly on individual CHV1 strain and on the combination of host and virus genomes, rather than on the virus subtype.

PT348 DncV synthesizes cyclic GAMP and regulates biofilm formation and motility in Escherichia coli ECOR31

Fengyang Li[1], Annika Cimdins-Ahne[1,2], Manfred Rohde[3], Lothar Jänsch[4], Volkhard Kaever[5], Manfred Nimtz[4], Ute Römling[1]

[1]*Karolinska Institutet, Department of Microbiology, Tumor and Cell Biology (MTC), Stockholm, Sweden*
[2]*University of Münster, Institute of Hygiene, Münster, Germany*
[3]*Helmholtz Centre for Infection Research, Central Facility for Microscopy, Braunschweig, Germany*
[4]*Helmholtz Centre for Infection Research, Cellular Proteomics, Braunschweig, Germany*
[5]*Hannover Medical School, Research Service Centre Metabolomics, Hannover, Germany*

Background: Cyclic dinucleotides (cDNs) act as intracellular second messengers, modulating bacterial physiology to regulate the fundamental life style transition between motility and sessility commonly known as biofilm formation. cGAMP, synthesized by the dinucleotide cyclase DncV, is a newly discovered second messenger involved in virulence and chemotaxis in *Vibrio cholerae*.

Objectives: To investigate a potential role for DncV in cGAMP production and regulation of biofilm formation and motility in the animal commensal strain *E. coli* ECOR31.

Methods: The rdar morphotype visualized on LB without salt agar plates was used as a model to assess biofilm formation. Motility was measured on soft agar medium. DncV was purified for enzymatic assay, followed by TLC assay to detect the catalytic activity. The enzymatic products were identified by MS and ESI-MS/MS. *In vivo* produced cDNs were extracted and detected by LC-MS/MS.

Results: ECOR31 expresses a semi-constitutive temperature-independent rdar morphotype on agar plates characterized by the extracellular matrix components cellulose and curli fimbriae, which requires activation by the major biofilm regulator CsgD and c-di-GMP signaling. In contrast, DncV negatively regulates the rdar morphotype and cell aggregation via down-regulation of *csgD* mRNA steady state level. Furtheron, DncV sequentially promotes and inhibits adhesion to the abiotic surface after 24 h and 48 h of growth, respectively. DncV also suppresses swimming and swarming motility post-transcriptional of class 1 flagella regulon gene *flhD*. Purified DncV produced different cDNs, c-di-GMP, c-di-AMP, unknown product(s), and the dominant species 3’3’-cGAMP. *In vivo* only 3’3’-cGAMP concentrations were elevated upon short-term overexpression of *dncV*.

PT349 Colony variation in K. pneumoniae reveals spontaneous capsule loss

Adriana Chiarelli[1], Nicolas Cabanel[2], Remy Bonnin[3], Philippe Glaser[2]

[1]Institut Pasteur, Microbiology, Paris, France
[2]Institut Pasteur
[3]Faculté de médecine Université Paris-Sud, Service de Bactériologie, Hôpital de Bicêtre, Le Kremlin-Bicêtre, France

Background: *Klebsiella pneumoniae* (Kp) is a leading cause of intractable multidrug-resistant (MDR) infections in hospitals. Most of the Kp clinical isolates produce capsule (CPS) as a major virulence factor. CPS-producing isolates exhibit opaque and mucoid colonies. Extensive recombination events at CPS locus are frequent and can generate capsule loss within *Klebsiella* spp. However, little is known about capsule switching in a bacterial population.

Objectives: Our objectives were to characterize heterogeneous populations of CPS+ and CPS- among carbapenemase-producing Kp (CPKp) and to study events causing spontaneous CPS loss, their frequency and to characterize colony variants.

Methods: Colony variants were distinguished by their morphology. Whole genome comparison was used to infer mutations causing phenotypic differences. CPS was quantified by uronic acid assay and visualized by India Ink staining. The frequency of CPS variation on tryptic-soy agar (TSA) was determined. Antimicrobial susceptibility testing (AST) and biofilm assays were performed to unveil putative differences in resistance.

Results: CPS spontaneous loss most frequently results of IS elements hopping into essential genes for capsule synthesis. Frequency of mutations on plates was strain dependent and increased in a density-dependent manner. Little or no capsule was produced by the CPS- variant. Phenotypic differences, including carbapenem susceptibility and *in vitro* biofilm formation were observed between the two variants. As the colony opacity differences in stored isolates may be undetected, researchers may be unconsciously working with mixed populations. This could be problematic for many studies, especially those involving virulence and resistance.

PT350 A new family of Staphylococcus aureus phages isolated from pattens with cystic fibrosis

Mercedes Cervera-Alamar[1], Migle Ziemyte[1], Patricia Bernabé Quispé[1], Mª Ángeles Tormo-Mas[1]

[1]*Health Research Institute Hospital La Fe, Valencia, Spain, Sever Infection Group, Valencia, Spain*

Background: Cystic fibrosis (CF) cause persist lung infection. *Staphylococcus aureus* is one of the earliest bacteria detected in children with CF. The variety of different virulence factors of *S. aureus* may increase morbidity and mortality rates of CF patients and their mobile genetic elements (MGEs) play an important role in adaptation to selective pressured imparted by the human host. Bacteriophages are MGEs that seem to have the greatest impact on staphylococcal diversity and evolution. Phage transduction allows the acquisition of virulence factors and antibiotic resistance.

Objectives: To characterize bacteriophages present in *S. aureus* strains isolated from CF patients, as well as to determine the mobilization mechanism of these MGEs.

Methods: Bacteriophage classification in 200 *S. aureus* isolates from 118 CF patients.

Sequencing and bionformatic analysis of 19 selected strains.

Construction of *S. aureus* mutants.

Results: 77% of these strains contained bacteriophage with integrase type III and 17 of the sequenced strains had conserved packaging module that was different from previously described phages. In all of the cases, phages contained TerL, endonuclease HNH and a hypothetical protein that might be a new TerS. Deletion of this protein do not affect phage DNA replication but completely eliminated phage packaging and infectivity. Morphological changes in phage structure were observed in this mutant. *cos*-site cleavage by this HNH-TerS-TerL complex was demonstrated. Finally, it was determined the role of these phages in mobilization and transference of virulence factors that might lead to better adaptation of these strains in patients with pulmonary infections in a worldwide.

PT351 peptidic inhibitors of toxin -anti toxin system mazef in staphylococcus aureus

Racheli Tamir[1]

[1]*Faculty of Agriculture, Food and Environment Robert H.. Smith, food science and biochemistry , Rehovot, Israel*

Background: *Staphylococcus aureus* (S. aureus) is a gram-positive bacteria that has been listed as a leading bacterial agent of a series of hospital and community acquired infections, with high priority for research and development of new antibiotics. The failure to eradicate *S. aureus* in clinical treatments is linked to the formation of persister cells. Persister cells are a small subpopulation of cells tolerant toward antibiotic by entering a state of dormancy or growth-arrest. Reducing or preventing growth arrest and the presence of persister cells may abrogate chronic infections.

Toxin-antitoxin (TA) systems are one of the modules that were proposed to be involved in the generation of persister cells. MazEF is one of three TA type II systems that were found in Methicillin resistance *Staphylococcus aureus* (MRSA). Disrupting TA complex formation may offer a prime opportunity for the development of selective antibacterial compounds since no TA mammalian homologs are known

Objectives: We explore the role of MazEFsa system in persister cells and biofilm formation by studying the interactions of MazEFsa Toxin-antitoxin complex.

Results: We have expressed and purified recombinant and active MazEF*sa* proteins and developed an *in-vitro* assay to test the catalytic activity of the proteins . We are focusing on designing selective peptides that aim to inhibit TA complex formation, to release the toxin to induce bacterial cell death. Our findings might open new direction in the design of novel antimicrobial agents that will be combined with the conventional antibiotics.

PT352 The Effects of Bacterial Cell Walls and Their Components on the Antimicrobial Activity and Structural Organization of Amyloidogenic Proteins of Bacillus thuringiensis Forming Functional Amyloids

Alla Polyanskaya[1], Guo Danyang[2], Alexander I. Netrusov[1], Tatiana G. Yudina[1]

[1]*Lomonosov Moscow State University, Russian Federation*
[2]*Institute of Agricultural Sciences and Technology, Shandong Academy of Agricultural Sciences, China*

Background: Amyloid fibrils (AFs) represent the unique quaternary structure of proteins and peptides. They normally demonstrate high antimicrobial activity. Many AFs playing key roles in the organism's adaptation to the environmental conditions are characterized as functional amyloids (FAs). We have previously demonstrated that parasporal crystal proteins of a number of enthomopathogenic bacterium *Bacillus thuringiensis* (Bt) subspecies are amyloidogenic proteins (APs) and belong to FAs.

Objectives: The purpose of our work was to study the effects of bacterial cell walls and cell wall components on the antimicrobial activity (AA) and structural organisation of APs in a number of Bt subspecies.

Methods: APs of *Bacillus thuringiensis* subspecies *kurstaki, morrisoni, israelensis, novosibirsk, silo, amagiensis* and FAs formed by them were analysed. Conventional bacteriological techniques were used to study AA. Changes in the structural state of Bt APs in the presence of the bacterial cell walls and their components (peptidoglycans, teichoic acid, N-acetyl-D-galactosamine, and their combinations) were assesed by spectrofluorimetry (with thioflavin T) and by transmission electronmicroscopy. Molecular masses of APs was determined by PAGE.

Results: We have shown that incubation of APs of the analysed Bt subspecies with the preparations of bacterial cell walls and their components prior to measuring the APs' antimicrobial activity stimulates AF formation by the APs to a different extent. The observed stimulation causes an increase in the AA of the analysed proteins and evidences for the importance of the Bt AP interaction with the cell walls of test bacteria.

PT353 Finding the small fry - novel genes in Escherichia coli

Klaus Neuhaus[1], Sonja Vanderhaeghen[2], Barbara Zehentner[2], Sarah Hücker[2], Zachary Ardern[2], Scherer Siegfried[2]

[1]Core Facility Microbiome/NGS, ZIEL - Institute for Food & Health, Technische Universität München, Freising, Germany
[2]Chair for Microbial Ecology, Freising, Germany

Background: Despite a relatively high coding density, still not all coding genes have been discovered in bacteria. Methods used until recently had a bias for larger genes especially. In turn, this caused a further theoretic bias, e.g. databases typically do not accept entries of single genes shorter than 100 codons.

Objectives: Novel, less biased methods such as RIBOseq (ribosomal footprinting) allow scanning the complete genomes of bacteria for evidence of translation, enabling detection of unannotated genes. In this way, a more complete picture of bacterial adaptation to environmental and novel conditions, including infections, is gained.

Methods: RIBOseq was used to screen genomes of *Escherichia* strains for translation events.

Results: Several hundred novel genes have been detected and confirmed. Some very unsual overlapping protein-coding genes were found as well.

PT354 incidence and resistance of the mycobacterium tuberculosis strains, isolated from clinical specimens in university clinical hospital (uch) mostar, from 2013. to 2017

Maja Ostojic[1], Andrea Boskovic[1]

[1]*University Clinical Hospital, Mostar, Bosnia and Herzegovenia*
[2]*Faculty of pharmacy and biochemistry, Zagreb, Croatia*

Background: *Mycobacterium tuberculosis* (MTb) remains a major global health problem, killing nearly 2 million patients around the world annually. Tuberculosis is quite present in Bosnia-Herzegovina, but over the past few years its incidence has declined.

Objectives: The aim of our study was to determine the incidence and resistance of the MTb strains, isolated in a five-year period in 3 cantons, gravitating towards the Microbiological laboratory of UCH Mostar.

Methods: A total of 11759 different samples were collected from the 3593 patients in UCH Mostar, from January 2013 to December 2017. They were assessed by direct specimen smear test, conventional culture, HAIN test, drug-susceptibility test (DST), as well as immunodiagnostics tests (Quantiferon TB Gold Test).

Results: One hundred and fifty nine (159) of cultured acid-fast bacteria were analysed by performing the GenoType MTBC and GenoType Mycobacteria CM/AS strip assays (Hain Lifescience). In each 159 smear and culture-positive specimens, all of them showed positive results with GenoType MTBC (100%). Only 1/159 (0.63%) strain showed resistance to rifampin and isoniazid. This has been confirmed by standard DST. Statistical analysis showed that positive test results for MTb throughout the given period were as following: 31/690 (4.49%) in 2013., 28/687 (4.07%) in 2014., 30/759 (3.95%) in 2015., 26/728 (3.57%) in 2016. and 44/729 (6.03%) in 2017. Quantiferon TB Gold Test was performed on 15 patients with smear and culture-positive specimens. Thirteen out of fifteen (86.66%) tests came out positive.

PT355 How the quorum-sensing communication of Gram- pathogenic bacteria is sensored and quenched by antagonistic Gram+ Actinobacteria

Corinne Barbey[2;3], Andrea Chane[2], Yvann Bourigault[2], Olivier Maillot[2], Annabelle Merieau[2;3], Mathilde Bouteiller[2], Amélie Beury-Cirou[4;5], Yoan Konto-ghiorghi[2], Marc Feuilloley[2], Virginie Gobert[4;5], Xavier Latour[2;3]

[1]*University of Rouen, Laboratory of Microbiology Signals and Microenvironement, Mont-Saint-Aignan, France*
[2]*Laboratory of Microbiology Signals and Microenvironment, Normandy University, Evreux, France*
[3]*Structure Fédérative de Recherche Normandie Végétale 4277 (NORVEGE)*
[4]*Seeds Innovation Protection Research and Environment (SIPRE), Bretteville du grand Caux, France*
[5]*French Federation of seed potato growers (FN3PT/RD3PT), Paris, France*

Background: The Gram positive *Rhodococcus erythropolis* disrupts virulence both of human and plant Gram negative pathogens by catabolizing their N-acyl-homoserine lactones (AHLs). This quorum-quenching activity requires the expression of the quorum-sensing signal degradation (*qsd*) operon, which encodes the lactonase QsdA and the fatty acyl-CoA ligase QsdC, involved in the catabolism of lactone ring and acyl chain moieties of signaling molecules, respectively.

Objectives: Surprisingly, this rhodococcal biocontrol activity is induced not by the invasion step or the massive presence of the pathogen, but only by its quorum-sensing based communication. Therefore, understanding the regulation mechanism which governs the induction of *qsd* operon expression leads to improve subtly clinical therapies and biocontrol strategies against pathogens.

Methods: Interactomic (*i.e.* molecular docking and EMSA), transcriptional and mutagenesis approaches coupled with confocal microscopy observations were successfully used to demonstrate the role of QsdR, a TetR-like family repressor, in the regulation of *qsd* operon expression.

Results: This transcriptional regulatory mechanism consists of the binding of the common part of AHL signaling molecules, the homoserine lactone ring, to the effector-receiving domain of QsdR, preventing a physical binding of QsdR to the *qsd* promoter region. To our knowledge, this is the first evidence revealing quorum signals as inducers of the suitable quorum-quenching pathway, confirming this QsdR protein as a lactone sensor. This regulatory mechanism designates the *qsd* operon as encoding a global disrupting pathway for degrading a wide range of signal substrates, allowing the broad spectrum anti-virulence activity observed (Barbey et al., 2018; Chane et al., 2019).

PT356 Studies on a heat stable beta-lactamase, which is produced by Bacillus cereus, inactivates penem antibiotics

Ritsuko Kuwana[1], Ryuji Yamazawa[1], Tatsuyuki Yamashita[2], Koichi Honke[2], Kiyoshi Ito[1], Hiromu Takamatsu[1]

[1]Setsunan University, Pharmaceutical Science, Hirakata, Japan
[2]Kochi University, Japan

Background: Antibiotics are used for the medical care, the domestic animals growth, and the control of health administration. Some antibiotics exposed in the environment are stable and keep the activities, which potentially causes the potential ecological and human health issue. The penem antibiotics, which are hardly to be degraded, are frequently used in Japan.

Objectives: In order to reduce the antibiotics pollution, we aimed to isolate soil bacteria that produce meropenem digestion enzymes.

Methods: Genome sequence analysis reveals that the some Bacillus species potentially produce antibiotic degradation enzymes. We screened Bacillus strains that can digest the meropenem from Biological Resource center in Japan and the soils.

Results: We found three natural isolates, SUBC1002, SUBC1008, and SUBC1010. We analyzed that they are included in B. cereus group. The MICs of meropenem were 2.5, 3.3, 3.4 mg/l, respectively. We found that the supernatant of B. cereus strains after the heat treatment at 80 ℃ was also effective to degrade the meropenem. We resolved the proteins included in the supernatant of B. cereus strains by SDS-PAGE and found major two bands with the molecular mass of 28-kDa and 33-kDa bands. We determined their amino acid sequences by LC-MS/MS. The 28-kDa protein corresponded to the B. cereus 5/B/6 beta-lactamase II. The 33-kDa protein corresponded to the B. cereus 5/B beta-lactamase I. These beta-lactamases belong to the different protein family and highly conserved among B. cereus group. These results suggested that some beta-lactamases produced by B. cereus strains potentially digested the meropenem.

PT357 Antimicrobial activities and genome insights of a novel Streptomyces strain isolated from rhizosphere soil of turmeric

Nareeluk Nakaew[1], Sineenart Sengyee[2], Worarat Kruasuwan[3], Narisara Chantratita[2], Rungroch Sungthong[4]

[1]*Naresuan University, Department of Microbiology and Parasitology, Faculty of Medical Science, Phitsanulok, Thailand*
[2]*Mahidol University, Department of Microbiology and Immunology, Faculty of Tropical Medicine, Bangkok, Thailand*
[3]*Kasetsart University, Department of Genetics, Faculty of Science, Bangkok, Thailand*
[4]*University of Glasgow, Infrastructure and Environment Research Division, School of Engineering, Glasgow, United Kingdom*

Antimicrobial resistance (AMR) is a global crisis, hampering the medical treatment of infectious diseases. Call for novel medicines with powerful functions to defeat AMR problem is urgent and ongoing. It is conceivable that various microbes are the producers of several antibiotics, but many of them are hidden in nature and yet waiting for exploration. With the aim of supplying new microbial cell factories for the biosynthesis of novel bioactive compounds, we isolated actinobacteria from the rhizosphere soil of turmeric. Among all, *Streptomyces* sp. TM32 showed antimicrobial activity against both human pathogens (e.g., methicillin-resistant *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Candida albicans*, etc.) and several phytopathogenic fungi (e.g., *Alternaria* sp., *Colletotrichum* sp., *Pyricularia* sp., *Rigidoporus* sp., etc.). Interestingly, even the antibiotic cocktail derived from strain TM32 were heated by autoclaving at 121°C and 15 psi for 15 min, its antimicrobial activities remained active. Draft genome analysis revealed that the strain's genome size is 7,343,383 bp (N_{50} = 5,559,518 bp), which closely related to *Streptomyces sioyaensis*, supported by the average nucleotide identities of 96.66 – 97.02%. The genome contained 73 contigs and 6,462 coding sequences, while some of which encoding several unknown polyketide antibiotics and enzymes involved with plant growth-promoting functions (e.g., indole-3-acetic acid production, phosphate solubilization, nitrate reduction, etc.). Based on either *in vitro* or *in silico* DNA-DNA hybridization, strain TM32 belonged to the same genomic species with *S*. *sioyaensis* but different subspecies. We believe that this novel *Streptomyces* strain would be a promising source for the discovery of new and potent antimicrobial substances.

PT358 biological re-purposing of fda-approved drugs as potent antibiofilm agent

Nidhi Verma[1], pankaj goyal[2], janmejay pandey[3]

[1]*central university of rajasthan, biotechnology, AJMER, India*
[1]*central university of rajasthan, biotechnology, AJMER, India*
[3]*central university of rajasthan, AJMER, India*

Background: Microbial Biofilms are multicellular communities of microbes, enclosed in self produced extra cellular matrix. These microbial communities render several advantages; on one hand, biofilms enable a variety of interactions amongst the bacterial cells residing within; on the other hand, it also enables resistance/ protection against unfavorable environmental conditions. *Bacillus subtilis* is a model organism of choice for studies on bacterial biofilm formation and its inhibition; the biofilm matrix in *B. subtilis* is mainly composed of exopolysaccharides, extracellular DNA and amyloid-like fibers making protein viz., TasA and TapA.

Objectives: In this study we applied *in silico* and *in vitro* approaches to screen FDA approved drugs to repurpose them for biofilm inhibition.

Methods: The present study was carried out to with homology modelling of *Bacillus* biofilm associated amyloid-like fibers making protein viz., TasA and TapA. Subsequently, the FDA approved drugs from ZINC database were used for virtual screening as potential inhibitors of the proteins for development of antibiofilm agent(s). The antibiofilm activity of selected compound was evaluated and ascertained in standard biofilm formation assays.

Results: Amongst the 3180 FDA-approved Drugs, the top 10 drug like molecules showed extra precision Glide score from -8.351 to -7.149 and mmGBSA binding energy from -105.374 to -92.929 Kcal/mol. In *in vitro* assays, the top selected compound showed biofilm inhibitory concentrations over a range of 6.25 - 25 µg/ml. The findings indicate that the identified ligands were stable in the active sites of TasA and TapA proteins and having antibiofilm activity against *B. subtilis.*

PT359 ARE-ABCF protein Sco0636 from Streptomyces coelicolor plays key role in resistance to tiamulin

Ludmila Veselá[1;2], Markéta Koběrská[1], Nikola Pinďáková[1], Gabriela Balíková Novotná[1]

[1]*The Czech Academy of Sciences, Institute of Microbiology v. v. i., Vestec, Czech Republic*
[2]*Charles University in Prague, Faculty of Science, Department of Genetic and Microbiology, Prague, Czech Republic*

Background: The ATP-binding cassette protein family F (ABCF) comprises largely uncharacterized but widely distributed ATPases which all seem to have a general role in ribosome-associated processes comprising regulation and resistance to clinically important ribosome-targeted antibiotics. ABCFs conferring resistance (ARE-ABCFs) are present in many clinically important pathogenic bacteria. They confer resistance through the antibiotic dissociation from binding site by interaction with ribosome.

Objectives: Many ABCFs from *Streptomyces* are not well characterized except for some from biosynthetic clusters. Therefore we tried to characterize ARE-ABCFs Sco0636 from model actinomycete *Streptomyces coelicolor* M145 which is outside clusters. For comparison we chose its homolog LmrC from lincomycin biosynthetic cluster of *Strepomyces lincolnensis* conferring lincosamide resistance.

Methods: To elucidate the contribution of Sco0636 to resistance in *S. coelicolor*, mutant strain with deleted *sco0636* was prepared by the published PCR-targeting method. Minimal inhibitory concentration to lincomycin, clindamycin, pristynamycin IIA, erythromycin and tiamulin was determined by agar dilution method.

Results: Sco0636 is responsible for high level of resistance to pleuromutilin antibiotic tiamulin. Only small changes in resistance to lincosamides and streptogramin A were detected. ARE-ABCFs confer resistance to antibiotic which can induce production of blue redox-active antibiotic actinorhodin. Due to this ability, we decided to test the influence of Sco0636 and LmrC to actinorhodin production. Sco0636 probably decreases effect of tiamulin to induction of actinorhodin production. We could see similar effect for LmrC and clindamycin. That led us to conclusion that ARE-ABCFs change actinorhodin production through conferring resistance. This project was supported by Charles University Grant Agency (1767418).

PT360 Stitching together signalling pathways using protein-protein interactions involving RpfR in Burkholderia cenocepacia H111

Rebecca Shilling[1], Elisabeth Steiner[1], Tim Tolker-Nielsen[2], Leo Eberl[3]

[1]University of Zurich, Department of Microbiology, Zürich, Switzerland
[2]Copenhagen University, Department of Immunology and Microbiology, København, Denmark
[1]University of Zurich, Department of Microbiology, Zürich, Switzerland

Background: RpfR is the receptor for *Burkholderia* diffusible signalling factor (BDSF) in *Burkholderia cenocepacia* H111. Deletion of RpfR affects behaviours including biofilm formation, motility and virulence. Current models rely on RpfR controlling cellular behaviour via its phosphodiesterase domain to decrease c-di-GMP levels. However, protein-protein interactions might manipulate downstream signals by altering protein conformation or by creating a very localised c-di-GMP pool. There is no described feedback mechanism between the BDSF synthase RpfF and RpfR.

Previous work has found that RpfR and RpfF co-localise in the cell and determining physical interactions could help to elucidate the feedback mechanism. BerB is a c-di-GMP binding, RpoN-dependent transcription factor involved in biofilm formation. An RpfR-BerB interaction would form a local pool of c-di-GMP giving control over costly biofilm formation.

Objectives: To fit RpfR into the known H111 signalling pathways by its protein-protein interactions.

Methods: The bacterial two hybrid system and β-galactosidase assays were used to detect and confirm protein-protein interactions.

Results: RpfR- and RpfF were confirmed to interact, and this was narrowed to the N-terminal residues 1-95 of RpfR, supporting a physical rather than genetic feedback loop. A small interaction domain, in comparison to the total size of RpfR, could allow other interactions to occur simultaneously or separately. The interaction between BerB and RpfR was also confirmed and suggested to involve the N-terminus of BerB, revealing how BDSF-based signalling controls biofilm formation. These interactions increase our understanding of how the BDSF signalling system controls biofilm formation in *B. cenocepacia*.

PT361 Structure and function of the DNA recombination apparatus involved in bacterial transformation

Thomas Perry[1], Chiara Rapisarda[1], Remi Fronzes[1]

[1]*Institut Européen de chimie et de biologie, Structure and function of bacterial nano-machines, Pessac, France*

Homologous recombination (HR) is a reaction essential for maintaining genomic integrity and for generating genetic diversity. HR is a highly conserved mechanism which allows the exchange of nucleotide sequences between two DNA molecules. This process is involved in the natural genetic transformation which is widely distributed in bacteria, more than 80 bacterial species have been reported to be naturally transformable. But natural genetic transformation was originally discovered in *Streptococcus pneumoniae (Sp)* in response to environmental signals, and ever since the pneumococcus has served as a paradigm for this important phenomenon.

Transformation requires dedicated cytosolic proteins, some of which have been characterized only recently, for the processing of internalized single-stranded DNA (ssDNA) fragments into recombination products. But many steps of this process remain unknown. It's in this context that my investigation takes place, in fact my research project aims to study, by cryo-microscopy, the interaction between the recombinase *S. pneumoniae* $RecA_{Sp}$ and the DNA. RecA, ATP and single-stranded DNA (ssDNA) form an helical filament that binds to double-stranded DNA (dsDNA), searches for homology, and then catalyzes the exchange of the complementary strand, producing a new heteroduplex.

Here, we report a structure for the $RecA_{Sp}$-ssDNA and the $RecA_{Sp}$-dsDNA complexes at 3.9Å and 3.8Å resolution respectively, using electron cryo-microscopy. $RecA_{Sp}$ encases ssDNA and dsDNA in a helical filament with a rise of 15.38Å and 14.97Å respectively, and with a twist of 58°. We show also, using these structures, why $RecA_{Sp}$ catalyzes ATP-dependent strand-exchange up to 3-fold more efficiently than *Escherichia coli* RecA protein.

PT362 structure-function analysis of a bacterial spirosomes

Pauline Pony[1;2], Esther Marza[1;2], Remi Fronzes[1;2]

[1]*European Institute of Chemistry and Biology, Pessac, France*
[2]*CNRS - UMR 5234 - MFP, structure and function of bacterial nano machines*

Background: Acetaldehyde–alcohol dehydrogenase (AdhE) enzymes are responsible for the conversion of acetyl-CoA to ethanol *via* an acetaldehyde intermediate during ethanol fermentation in anaerobic environment. This two-step reaction is associated to NAD^+ regeneration, essential for the glycolysis pathway. The bifunctional AdhE enzyme is well-conserved in all bacterial kingdoms and also in more phylogenetically distant microorganisms. AdhE is well-studied for its involvement in biofuel production, but as well for its role in bacterial pathogenicity. This enzyme is commonly found as an oligomeric form called spirosomes. Despite the great interest in the AdhE enzyme, the structure and function of these filaments remain unknown since their discovery in the 70's.

Objectives: The purpose of this study is to investigate the role of spirosomes using a structure-function analysis.

Methods: Cryo-electron microsocopy was used to solve the structure of purified *Escherichia coli* spirosomes. Mutagenesis of AdhE sequence was performed in order to disrupt the filamentation. Using absorbance spectrophotometry, the enzymatic reaction was monitored for the wild-type and the mutant protein.

Results: Here, we report for the first-time the structure of a bacterial spirosome. The *Escherichia coli* acetaldehyde – alcohol dehydrogenase polymer structure was solved at 3.9Å resolution using cryoEM in an extended conformation. Using a structure-function approach, conserved interfaces into this dynamic spirosome are described. In addition, the enzymatic activities of both native and disrupted spirosomes are discussed. Finally, it is suggested that spirosomes could be the predominant form during alcoholic fermentation, preventing the diffusion of the toxic reactive intermediate, into the cytoplasm of the cell.

PT363 A novel amino acid substitution, Ser83Ile provides high-level quinolone resistance to Salmonella Enteritidis DNA gyrase without compromising enzyme activity

Kentaro Koide[1], Lai Lai San[2], Chie Nakajima[1;3], Yasuhiko Suzuki[1;3]

[1]Hokkaido University Research Center for Zoonosis Control, Division of Bioresources, Sapporo, Japan
[2] Ministry of Health and Sports, Department of Medical Research, Myanmar
[3]Global Station for Zoonosis Control, Global Institution for Collaborative Research and Education (GI-CoRE), Hokkaido University, Japan

Background: A novel amino acid substitution, Ser83Ile, on GyrA was found in quinolone resistant *Salmonella* Enteritidis in some Asian countries. GyrA is one of the subunits constituting DNA gyrase, and it is known that quinolone resistance is obtained by amino acid substitution here. The substitution to Ile (ATC) needs a secondary base substitution from a primary substitution of Ser (TCC) via Phe (TTC) at position 83 on GyrA. It is likely that Ile might provide some advantages to quinolone resistance and/or the enzyme activity of DNA gyrase.

Objectives: We examined the direct impact of Ser83Ile on DNA gyrase comparing to other amino acid substitutions.

Methods: Recombinant wild type (WT) and seven mutant DNA gyrases having Ser83Ile, Ser83Phe, Ser83Thr or other frequently reported amino acid substitutions were expressed in *Escherichia coli* and purified by affinity chromatography. The enzyme activity was assessed by measuring over time the amount of circular DNA supercoiled by purified DNA gyrase. Quinolone resistance was evaluated by the concentration of ciprofloxacin which was required to inhibit the supercoiling activity by 50% (IC_{50}).

Results: In the result, the supercoiling activity of Ser83Ile DNA gyrase was not decreased comparing to WT. However, IC_{50} for Ser83Ile mutant DNA gyrase was much higher than WT and other mutant DNA gyrases. That is, Ser83Ile led to an increase in quinolone resistance without decreasing the enzyme activity. It can be concluded that *S.* Enteritidis possessing Ser83Ile DNA gyrase shows high quinolone resistance and it has a high possibility to spread worldwide.

PT364 Indole Regulation of Cytoplasmic pH in E. coli: Insights into Bacterial Memory and Antibiotic Resistance

Ashraf Zarkan[1], Santiago Cano Muniz[2], David Summers[3]

[1]*University of Cambridge, Genetics, Cambridge, United Kingdom*
[2]*University of Cambridge, MRC Laboratory of Molecular Biology, Cambridge, United Kingdom*
[1]*University of Cambridge, Genetics, Cambridge, United Kingdom*

Background: Indole is an aromatic molecule with diverse signalling roles. Two modes of signalling have been described: persistent and pulse signalling. The latter is illustrated by the brief but intense elevation of intracellular indole during stationary phase entry.

Objectives: Indole pulse plays a crucial role in the regulation of *E. coli* cytoplasmic pH, which potentially provides a degree of protection against specific antibiotics.

Methods: Cytoplasmic pH of *E. coli* was measured using a plasmid-encoded mCherry-pHluorin fusion protein. The fluorescence is either pH-sensitive or pH-insensitive, for pHluorin and mCherry, respectively. Thus, mCherry was used for normalisation.

Results: *E. coli* cells growing under conditions where no indole is produced maintain their cytoplasmic pH near 7.8. In contrast, under conditions permitting indole production, the cytoplasmic pH is maintained near 7.2. The pH regulation results from pulse, rather than persistent, indole signalling. The relevant property of indole in this context is its ability to conduct protons across the cytoplasmic membrane. Although the pulse is brief, its effect remains as a “memory” to maintain the correct cytoplasmic pH until entry into the next stationary phase. The pulse is probably influencing the distribution of membrane proteins, with the new distribution serving as the memory repository. Additionally, we show that the loss of indole production in *E. coli* reduces the formation of antibiotic persisters, which is a sub-population (<1%) of genetically-sensitive cells that survive antibiotics by becoming dormant. We have explored whether the indole-mediated regulation of cytoplasmic pH may explain the reduction in persisters formation.

PT365 A Possible Additional Site for Chromosome Dimer Resolution in E. coli

Cameron Croft[1], Cindy Macpherson[1], David Summers[1]

[1]*University Of Cambridge, Department of Genetics, Cambridge, United Kingdom*

Background: Dimer formation is a serious threat to the stable maintenance of ColE1-like plasmids. Dimers form infrequently by homologous recombination but accumulate rapidly by having two origins of replication. This results in elevated plasmid loss and a reduction in host cell growth rate. Plasmid dimers are resolved to monomers by the XerCD recombinase plus accessory proteins ArgR and PepA, acting at the *cer* recombination site. The circular chromosome of *E. coli* also forms dimers infrequently, and consequent failure of chromosome partition results in filamentation, SOS induction, and failure of cell division. Site-specific recombination is required for dimer resolution during cell division by XerCD acting at the *dif* (deletion induced filamentation) site near the *E. coli* chromosome terminus. ArgR and PepA proteins are nonessential, but the septum-associated protein FtsK is necessary for dimer segregation, suggesting the XerCD/*dif* complex interacts with division septums. Our preliminary work had revealed homology between *cer* and a 170-bp chromosomal site (*tcs*, terminal region *cer*-like site) 1.2 min from *dif*.

Objectives: Determine whether *tcs* is capable of recombination for resolution of dimers.

Methods: The *tcs* site and surrounding region was cloned into plasmids, dimer plasmids were purified and then assayed for *tcs*-mediated recombination.

Results: We demonstrated that a construct with a 500-bp *tcs* insert supports XerCD-mediated recombination, whereas smaller constructs were recombination-deficient. The absence of plasmid monomerization in mutant strains indicates that ArgR and PepA are required for recombination. Given the similarities between *cer*, *tcs*, and *dif*, these results suggest that *tcs* could facilitate chromosome dimer resolution.

PT366 investigating the fitness costs of mobile colistin resistance genes

Lois Ogunlana[1]

[1]*Oxford, Department of Zoology, oxford, United Kingdom*

Background: Antibiotic resistance is a worldwide problem and one of the major scientific focuses currently. Recently, plasmid mediated mobile resistance to colistin (a last-resort antibiotic against gram-negative bacteria) via the mcr-1 gene has been discovered in E. coli and K. pneumoniae. However, expression of this gene imposes a major fitness cost on bacteria. Multiple mobile colistin resistance (MCR) gene variants and homologs have since been identified globally.

Objectives: My project aims to assess the fitness costs of these homologs and variants in order to determine if these costs are being alleviated.

Methods: To achieve this goal, we have been competing different MCR-1 variant genes in bacterium of identical genetic backgrounds in order to probe the individual effects of the gene on bacterial fitness.

Results: Preliminary results show that certain variants achieve higher levels of colistin resistance than the consensus mcr-1 gene with the caveat of increased fitness costs. Other variants show lower colistin resistances but have increased fitness in the absence of the antibiotic. Results also demonstrate the non-linearity between MCR expression and colistin resistance.

PT367 Biochemical and structural study of colicin M and its orthologues targeting peptidoglycan biosynthesis. Potential use as novel antibiotics

Libera Latino[1], Dimitri Chérier[1], Delphine Patin[1], Didier Blanot[1], Thierry Touzé[1], Dominique Mengin-Lecreulx[1], Hélène Barreteau[1]

[1]*Institute of Integrative Biology of the Cell, Orsay, France*

Background: Colicins are bacteriocins produced by some *Escherichia coli* strains in order to kill competitors. All known colicins share the same structural organization in three domain, each of them being devoted to a specific function: translocation through the outer membrane, binding to a specific receptor and toxicity, from N to C termini, respectively. Colicin M (ColM) is the bacteriocin known to interfere with peptidoglycan biosynthesis leading to cell death. Several homologues of ColM produced by various bacteria such as *Pseudomonas*, *Burkholderia* and *Pectobacterium* have been recently identified. They exhibit the same mode of action as ColM and display cytotoxic activity towards a limited number of bacterial species.

Objectives: We propose the creation of chimeric bacteriocins in order to get potent antibacterials able to fight against various pathogenic bacterial species.

Methods: We will take advantage of the modular structure of lipid II-degrading bacteriocins to construct, through molecular engineering, chimeric bacteriocins capable of targeting different bacterial genera.

Results: We recently showed that the pectocin M1 (PcaM1, produced by *Pectobacterium carotovorum*) was able to kill *E. coli* cells once addressed to the periplasm of this species. In these conditions, PcaM1 and its isolated catalytic domain were able to catalyze the degradation of lipid II, leading to the arrest of peptidoglycan biosynthesis and cell lysis. To go further, we constructed chimeric colicins directed against *E. coli* and constituted of the N-terminal reception and translocation domains of ColM followed by the catalytic domain of PcaM1. My presentation will deal with our preliminary and encouraging results.

PT368 NMR-Metabolomics shows that BolA is an important modulator of Salmonella Typhimurium metabolic processes under virulence conditions

Gil Graça-Lopes[1], Gonçalo Graça[1], Susana Barahona[1], Ricardo N. Moreira[1], Luís G. Gonçalves[1], Cecília M. Arraiano[1]

[1]ITQB NOVA, Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Portugal, Oeiras, Portugal

Background: BolA is a widespread protein that can act as a global transcription factor. BolA production leads to significant stress-resistance physiological changes such as the induction of a spherical shape, reduction of outer membrane permeability and promotion of biofilm development. Furthermore, it has been shown that BolA increases the virulence of *Salmonella* spp, but further research effort is required to shed light on the pathways modulated by BolA.

Objectives: In this study, we resorted to untargeted ^{1}H-NMR metabolomics to understand the impact of different levels of *bolA* expression on the metabolic profile of *Salmonella* Typhimurium, under virulence conditions.

Methods: Three strains of *S*. Typhimurium were studied: a strain transformed with an empty pWSK29 plasmid (control); a *bolA* knockout mutant (Δ*bolA*) and a strain overexpressing *bolA* using pWSK29 as the expression vector (*bolA*$^{+}$). These strains were grown in a minimal virulence-inducing medium and cells were collected at the end of the exponential and stationary phases. Cell metabolism was immediately quenched and intracellular metabolites were extracted. The extracts were analysed by NMR and multivariate and univariate statistical analysis were performed to identify significant alterations.

Results: By performing principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA) on ^{1}H-NMR data, we were able to discriminate between the metabolic profiles of these strains, revealing increased levels of acetate, valine, alanine, NAD^{+}, succinate, coenzyme A, glutathione and putrescine in *bolA*$^{+}$.

PT369 Reciprocal effect between genetic instability and oxidative stress for synthetic growth defect in budding yeast

Woo-Hyun Chung[1], Ji Eun Choi[1]

[1]*Duksung Women's University, College of Pharmacy, Seoul, Korea, Rep. of South*

Background: High-throughput analysis of synthetic lethal genetic interactions in yeast revealed that defects of *TSA1* or *SOD1*, encoding two reactive oxygen species (ROS)-detoxifying enzymes, impaired growth of DNA double-strand break (DSB) repair-deficient mutants. Therefore, SOD1 inhibition has been suggested as a promising approach for selective killing of *RAD54B*-deficient colorectal cancer cells.

Objectives: The *rad51 tsa1* and *rad51 sod1* double mutant strains were created to examine whether those genetic combinations display synthetic lethality and molecular mechanisms in charge of their cytotoxicity and drug sensitivity were investigated.

Methods: Cell viability and drug sensitivity were determined by spotting analysis. The rate of mutagenesis was measured by a forward-mutation assay. To measure genomic instability, fluorescence microscopy with GFP-fused proteins and western blot analysis were used. FACS analyses were used to determine cell cycle progression and to measure intracellular ROS levels.

Results: The *rad51 sod1* double mutant is not synthetic lethal but displays severely slow growth and synergistic sensitivity to both ROS- and DSB-generating drugs. The function of Sod1 regarding genome stability depends on Ccs1, suggesting the significance of its antioxidant activity for the interaction with Rad51. Although ROS-induced nuclear localization of Sod1 is independent of Rad51, Sod1 is involved in prevention of mutagenesis and DNA damage checkpoint activation together with Rad51. Inversely, lack of Rad51 elevates intracellular ROS level and aggravates cytotoxicity derived from Sod1 deficiency. Altogether, these results suggest that there is a significant and specific crosstalk between two major cellular damage response pathways, ROS signaling and DSB repair, for cell viability.

Fig. 1

Fig. 3

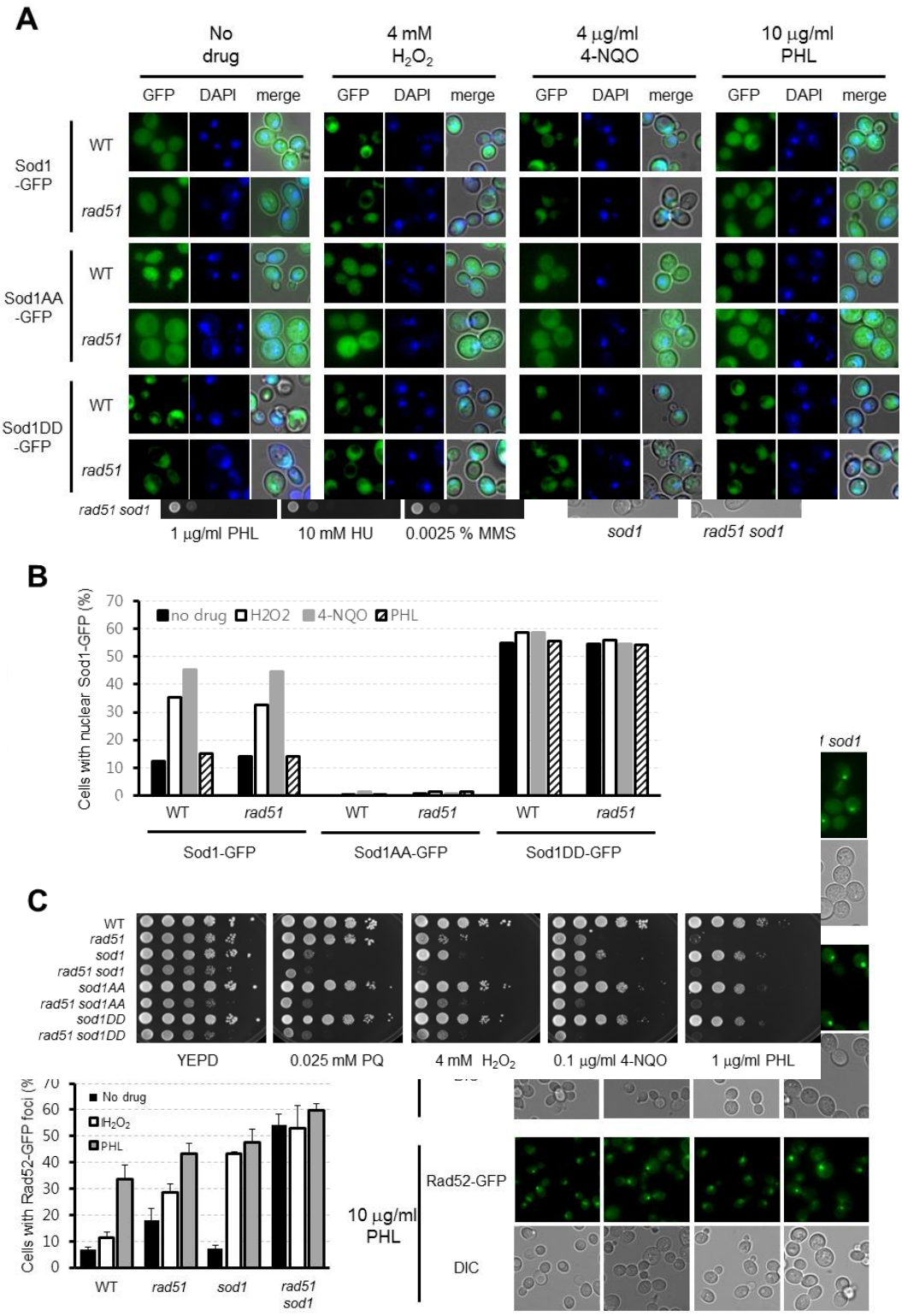
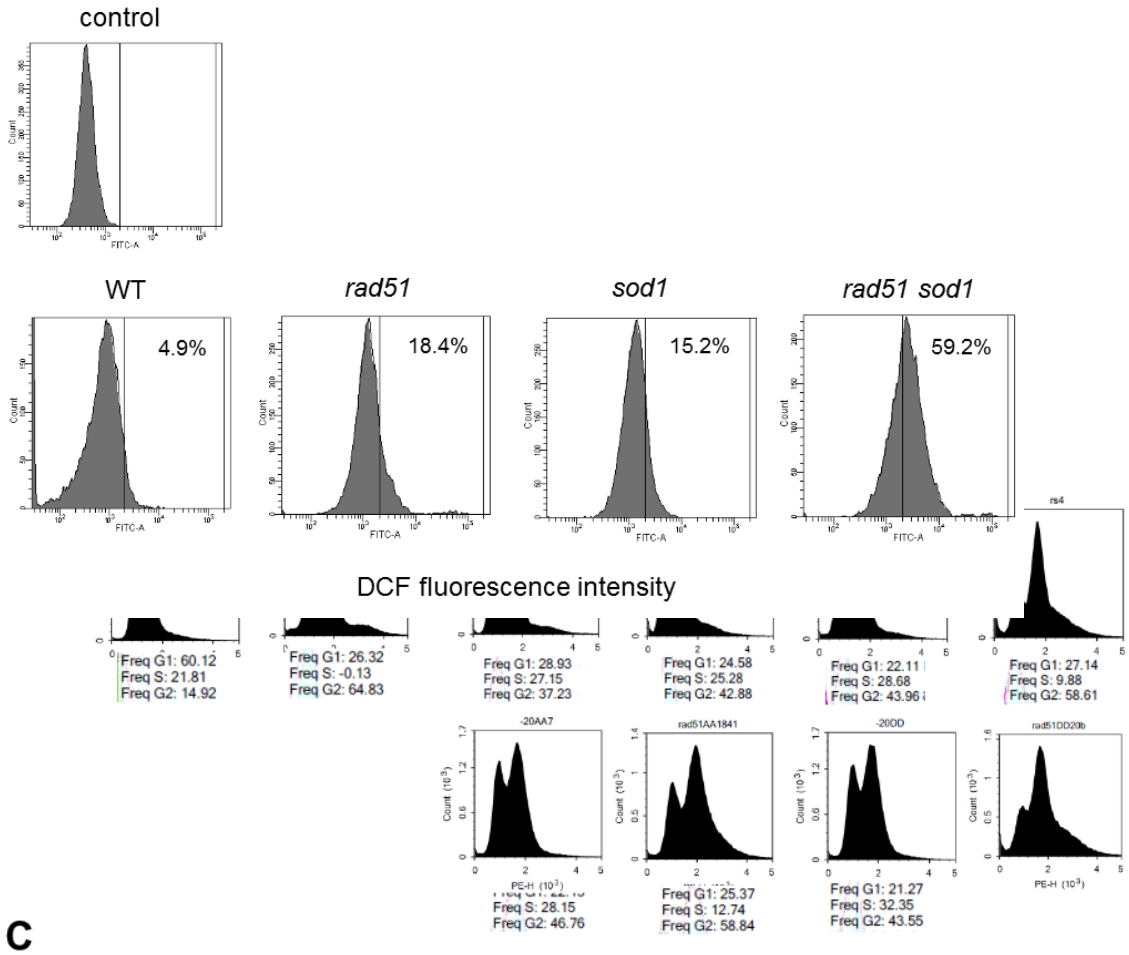
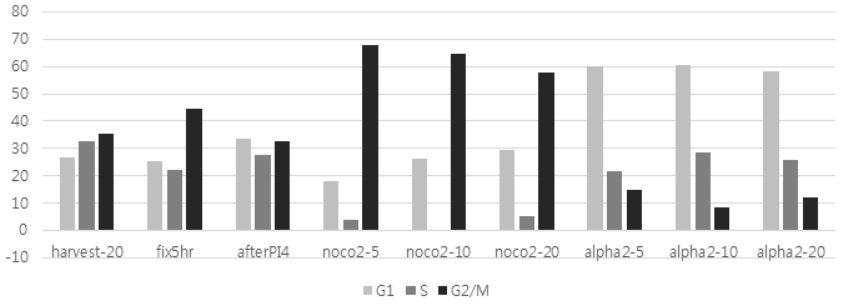

A
No drug
4 mM H_2O_2
4 μg/ml 4-NQO
10 μg/ml PHL
GFP DAPI merge
Sod1 -GFP
Sod1AA -GFP
Sod1DD -GFP
WT
rad51
rad51 sod1
1 μg/ml PHL
10 mM HU
0.0025 % MMS
sod1
rad51 sod1
B
no drug H2O2 4-NQO PHL
Cells with nuclear Sod1-GFP (%)
Sod1-GFP
Sod1AA-GFP
Sod1DD-GFP
C
WT
rad51
sod1
rad51 sod1
sod1AA
rad51 sod1AA
sod1DD
rad51 sod1DD
YEPD
0.025 mM PQ
4 mM H_2O_2
0.1 μg/ml 4-NQO
1 μg/ml PHL
No drug
H_2O_2
PHL
Cells with Rad52-GFP foci (%
10 μg/ml PHL
Rad52-GFP
DIC

Fig. 5


control
WT
rad51
sod1
rad51 sod1
4.9%
18.4%
15.2%
59.2%
FITC-A
Count
DCF fluorescence intensity
Freq G1: 60.12
Freq S: 21.81
Freq G2: 14.92
Freq G1: 26.32
Freq S: -0.13
Freq G2: 64.83
Freq G1: 28.93
Freq S: 27.15
Freq G2: 37.23
Freq G1: 24.58
Freq S: 25.28
Freq G2: 42.88
Freq G1: 22.11
Freq S: 28.68
Freq G1: 27.14
Freq S: 9.88
Freq G2: 58.61
-20AA7
rad51AA1841
-20DD
rad51DD20b
PE-H (10^3)
Freq S: 28.15
Freq G2: 46.76
Freq G1: 25.37
Freq S: 12.74
Freq G2: 58.84
Freq G1: 21.27
Freq S: 32.35
Freq G2: 43.55

C


80
70
60
50
40
30
20
10
0
-10
harvest-20
fix5hr
afterPI4
noco2-5
noco2-10
noco2-20
alpha2-5
alpha2-10
alpha2-20
G1
S
G2/M

PT370 the effect of cpx two component system on vibrio parahaemolyticus psd gene expression

Jui-An Chan[1], Chia-Yin Lee[2]

[1]National Taiwan University, Microbiology Laboratory, Agricultural Chemistry, Taipei, Taiwan
[1]National Taiwan University, Microbiology Laboratory, Agricultural Chemistry, Taipei, Taiwan

Background: *Vibrio parahaemolyticus* is a Gram-negative bacterium that thrives in marine or estuarine. Three major types of phospholipids are present in *V. parahaemolyticus*: 80% phosphatidylethanolamine (PE), 10% cardiolipin, and 5% phosphatidylglycerol. Phosphatidylserine decarboxylase (Psd) (EC 4.1.1.65) is an enzyme which catalyze the formation of PE. It plays a central role in the phospholipid metabolism. The Cpx two component system comprised of the histidine kinase CpxA and the response regulator, CpxR. It serves as a common stimulus response mechanism, thus allows microbes to sense and respond to diverse environments through a series of phosphorylation reactions.

Objectives: Psd is involed in phospholipid metabolism and alterations in the ratio of inner phospholipids. It synthesized the major phospholipid in *V. parahaemolyticus.* Therefore, we investigated the correlation of *psd* and Cpx two component system in this study.

Methods: Luminescence assay and real-time PCR were used to investigate whether *psd* was regulated by CpxR. ARF-TSS assay was used to confirmed the *psd* transcription start site. The interaction of *psd* promoter region with CpxR was examined by EMSA.

Results: We found that *psd* is expressed from the bacterial early-log phase (OD_{600} to 0.25). The *psd* gene expression increased 2-fold at Δ*cpxR*、Δ*cpxA*、Δ*cpxRA* mutants compared to the wild-type strain. CpxR repressed *psd* at mid-log phase (OD_{600} to 2.3). The transcription start site of *psd* was 48 nucleotides upstream of the start codon. No shift band was observed in the EMSA, we suggest *psd* is indirectly affected by CpxR.

PT371 investigating the fitness costs of mobile colistin resistance genes

Lois Ogunlana[1], stephan uphoff[2], Craig Maclean[1]

[1]*Department of Zoology, University of Oxford, United Kingdom*
[2]*New Biochemistry Building, Department of Biochemistry, United Kingdom*

Background: Antibiotic resistance is a worldwide problem and one of the major scientific focuses currently. Recently, plasmid mediated mobile resistance to colistin (a last-resort antibiotic against gram-negative bacteria) via the mcr-1 gene has been discovered in E. coli and K. pneumoniae. However, expression of this gene imposes a major fitness cost on bacteria. Polymorphisms in MCR-1 have been discovered in clinical and environmental bacterial strains, suggesting that MCR-1 may be evolving compensatory adaptations to offset the cost of colistin resistance.

Objectives: My project aims to assess the fitness costs of these homologs and variants in order to determine if these costs are being alleviated.

Methods: We have cloned 8 previously described variants of MCR-1 into a lab strain of E.coli, and we have measured the effect of these variants on colistin resistance and its cost using a combination of MIC assays and competitive fitness assays. We have been competing different MCR-1 variant genes in bacterium of identical genetic backgrounds in order to probe the individual effects of the gene on bacterial fitness.

Results: Our key finding is that some variants of MCR-1 achieve higher levels of colistin resistance than the mcr-1 gene with the caveat of increased fitness costs. Other variants show lower colistin resistances but have increased fitness in the absence of the antibiotic. These results show that a trade-off between antibiotic resistance and fitness is driving the rapid evolution of the MCR-1 resistance genes.

PT374 Identification of Antimicrobial Compounds in Zingiber Officinale

Ankith Maremanda[1], Eric Espinosa[1]

[1]*BioCurious, Santa Clara, United States*

Background: Resistance to antibiotics has rendered bacteria to be extremely dangerous to other organisms, through epidemics such as food poisoning, pneumonia, and chlamydia. As a result of these epidemics, the need for new antibiotics that will combat the rapid reproduction of antibiotic-resistant bacteria is more important than ever before.

Objectives: Zingiber Officinale (Ginger) has been used as a medicinal plant for generations, possessing alleged antimicrobial properties that have been seldom tested. The aim of this project was to empirically determine prove the validity of the existence of antimicrobial compounds in ginger and garlic in order to provide possible effective alternatives for the antibiotics that bacteria are gaining resistance to.

Methods: The primary methodology this experiment uses to elucidate the molecule possibly responsible for antibacterial properties in ginger is size-exclusion chromatography. This form of fractionation helps in identifying the relative size of the possible antimicrobial compounds, making them easier to identify. These fractionated compounds were added to bacterial solution, and effectiveness was gaged through the use of OD readings given by a spectrophotometer.

Results: Based on other assays performed apart from spectrophotometer readings (eg. boiling fractions with proteins), it was determined that fractions from ginger containing concentrations of DNA Gyrase Subunit-B and Violaxanthin de-epoxidase (found through mass spectometry) were effective in inhibiting bacterial growth. The antimicrobial properties of these enzymes were further proven by creating them in-vitro in higher concentrations and then directly exposing them to bacterial solution, in which bacterial growth was inhibited.

PT375 An integron-cassette capture device to detect resistance cassettes

Filipa Trigo da Roza[1], Alberto Hipólito[1], Rocío López-Igual[2], José Antonio Escudero[1]

[1]Facultad de Medicina Veterinaria, Universidad Complutense de Madrid, Molecular Basis of Adaptation Laboratory, Madrid, Spain
[2]CSIC y Universidad de Sevilla, Instituto de Bioquímica Vegetal y Fotosíntesis, Sevilla, Spain

Background: Integrons are genetic platforms that capture and stockpile resistance gene cassettes. These structures have played a major role in the rise of multidrug-resistant bacteria, harboring more than 130 different antibiotic resistance genes. Yet, despite their importance, our tools to detect integron cassettes are flawed or inefficient.

Objectives: Our aim is to develop a biotechnological tool to capture integron cassettes from DNA samples. We will use it to analyze in an unprecedented way the prevalence and circulation of resistance cassettes in all the fields of the One Health concept.

Methods and Results: We have reengineered a class 1 integron to serve as a capture device using the CcdB toxin as a blind reporter. The rationale here is that, by embedding a cassette integration site (*attI*) within *ccdB*, incoming cassettes will disrupt the toxin so that recombinants will survive upon repression of the antitoxin-encoding gene *ccdA*. Embedding a 57 bp-long *attI* site in the 320 bp-long *ccdB* gene, without disrupting its function is challenging. We have introduced the *attI* site in a flexible region between strands of the minor ß-sheet of CcdB. The killing activity of *ccdB::attI* upon repression of *ccdA* is similar to that of wild type *ccdB*, suggesting that the insertion of *attI* interferes minimally with the activity of the protein. As a control, we have introduced a variety of cassettes in *attI*, proving that the toxin is effectively disrupted. This provides a large dynamic range (10^7) for the detection of insertion cassettes using this tool.

PT376 Construction of an antibacterial tool based on mobilization by the conjugative system of the IncM plasmid pCTX-M3

Izabela Kern-Zdanowicz[1], Michał Dmowski[1]

[1]*Institute of Biochemistry and Biophysics, Warszawa, Poland*

Background: Conjugative plasmids are main players in horizontal gene transfer in gram-negative bacteria. They contribute to rapid dissemination of antibiotic resistance genes causing the crisis of antibiotic-resistant bacteria which urgently requires new solutions. The IncM plasmids are one of major families of resistance plasmid of clinically relevant *Enterobacteriaceae*. The range of recipients of their conjugation system is broad and comprises *Alpha-*, *Beta-*, and *Gammaproteobacteria* as well as representatives of *Firmicutes*. The expression of the conjugation system of the IncM plasmid pCTX-M3, is repressed in the presence of *orf35*- and *orf36*-encoded IncM-specific regulators. Here, we exploited the tailored pCTX-M3 conjugation system to deliver antimicrobial agents.

Objectives: On the basis of pCTX-M3 helper strains efficient in mobilization were constructed. They can be used in conjugation to introduce mobilizable plasmids encoding proteins toxic to recipients.

Methods: Genes encoding the conjugation system of pCTX-M3 were integrated into *Escherichia coli* chromosome to construct the helper strains efficient in mobilization of $oriT_{pCTX\text{-}M3}$-bearing plasmids. These strains were deprived of regulatory genes: *orf35* only or *orf35* and *orf36*. The mobilizable plasmid encoded Zeta (UDP-N-acetylglucosamine kinase), a toxin of the toxin-antidote system from the streptococcal plasmid pSM19035, previously shown to act on *E. coli cells*; the donor cells were protected by expressing Epsilon, the antidote.

Results: The constructed strains were shown to mobilize the control plasmid with the truncated *ζ* (*zeta*) gene at efficiencies more than 1 transconjugant/donor. When the Zeta-encoding plasmid was mobilized, a decrease by five orders of magnitude in transconjugants number was observed.

PT377 Growth promotion in Corynebacterium glutamicum by NCgl2986 gene overexpression encoding an amidase-like protein

Mia Fitria Utami[1], Yoshihiko Matsuda[2], Ayako Takada[3], Noritaka Iwai[1], Takashi Hirasawa[1], Masaaki Wachi[1]

[1]Tokyo Institute of Technology, School of Life Science and Technology, Yokohama, Japan
[2]Research Institute for Bioscience Products & Fine Chemicals, Ajinomoto Co., Inc., Japan
[3]Tokyo Institute of Technology, Biomaterials Analysis Division, Technical Department, Yokohama, Japan

Background: *Corynebacterium glutamicum* is a well-known bacterium that globally used to produce amino acid. It has unique cell wall organization composed of peptidoglycan, arabinogalactan, and mycolic acid. *C. glutamicum* possesses hydrolase NCgl2986 and we found that overexpression of NCgl2986 encoding an amidase-like protein resulted in cell growth promotion.

Objectives: Uncover the role of NCgl2986 in the growth promotion effect of *C. glutamicum*

Methods: During growth assay experiment, cells were grown on protein production MMTG medium. NCgl2986- or MurA-overexpressed cells were analyzed by SEM to determine the cell size precisely. Furthermore, to measure the activity of NCgl2986 enzyme, his-tagged NCgl2986 was purified.

Results: When cells were grown in protein production medium MMTG containing high concentration of glucose and neutralizing agent $CaCO_3$, we observed that not only turbidity but also dry cell weight was increased by NCgl2986 overexpression. Further analysis showed that this growth promotion resulted from the increase in cell volume and cell number. His-tagged NCgl2986 was purified but peptidoglycan hydrolyzing activity was not detected. Furthermore, MurA overexpression mimicked the NCgl2986 overexpression; active in rich medium, improved cell growth, and more susceptible to ampicillin. These results suggest that NCgl2986 functions as an activator by interacting with MurA protein, which involved in the first step of peptidoglycan precursor synthesis. Hence, NCgl2986 overexpression promotes the cell growth by enhancing the cell biomass synthesis.

PT378 Allosteric feedback inhibition of pyridoxine 5'-phosphate oxidase from Escherichia coli

anna barile[1], roberto contestabile[1], martino luigi di salvo[1], angela tramonti[2], francesco malatesta[1]

[1]*University of Rome, La Sapienza, Biochemical Sciences "A. Rossi Fanelli", Rome, Italy*
[2]*Institute of Molecular Biology and Pathology, National Research Council, Rome, Italy*

Background: In *Escherichia coli*, the synthesis of pyridoxal 5'-phosphate (PLP), the catalytically active form of vitamin B_6, takes place through the so-called deoxyxylulose 5-phosphate-dependent pathway, whose last step is the oxidation of pyridoxine 5'-phosphate (PNP) to PLP, catalysed by the FMN-dependent enzyme PNP oxidase (PNPOx). This is a catalytically sluggish enzyme that plays a pivotal role in controlling PLP intracellular homeostasis and bioavailability.

Objectives: PNPOx has been proposed to undergo product inhibition resulting from PLP binding at the active site. PLP also binds tightly at an allosteric site and in this way is sequestered from solvent, but at the same time made available to the apoenzymes that use it as cofactor, through a transfer process whose mechanism is unclear. Binding of PLP at the allosteric site has been reported to take place without inhibition of the enzyme activity. The possible location of this allosteric site has been indicated by crystallographic studies as two symmetric surface pockets present on the PNPOx homodimer, but never verified by other experimental means.

Methods: Kinetic measurements and equilibrium binding studies have been carried out on wild type and mutant PNPOx forms.

Results: We demonstrate that PLP inhibition has a mixed-type nature and results from binding of this vitamer at an allosteric site. The calculated binding stoichiometry of one PLP molecule per enzyme dimer suggests that the allosteric binding site probably does not coincide with that indicated by previous crystallographic studies. This novel interpretation of PNPOx feedback inhibition prompts further investigations on this enzyme.

PT379 Single-cell screening of microbes: following antibiotic resistance gene carriers

Lara Dutra[1], Reetta Penttinen[1], Ole Franz[1], Kimi Nurminen[1], Pauliina Salmi[1], Matti Jalasvuori[1], Marja Tiirola[1]

[1]*University of Jyväskylä, Department of Biological and Environmental Sciences, Jyväskylä, Finland*

Background: Microorganisms play many important roles in processes that shape life on our planet from nutrient cycles to health and disease in other living organisms. Metagenomics and amplicon sequencing have been widely employed in profiling entire microbial communities. While metagenomics presents an entire picture of the community's taxonomy and functions, amplicon sequencing allows following specific targets. There is, however, a growing interest for the development of new tools to enable single-cell screening of entire communities. Droplet microfluidics has emerged as a powerful tool for biological analysis, increasing test's sensitivity and specificity by reducing sample size. Droplet Digital PCR (ddPCR) is a well-known and commercially available system that uses droplet microfluidics to generate nanolitre-sized PCR reactions for target quantitation. Lately, droplet microfluidics has been used to distribute population of cells into small partitions.

Objectives: The aim of this work was to develop a method to screen for antibiotic resistance gene (ARG) carriers in heterogeneous sample.

Methods: The ddPCR microfluidics system was used to create partitions containing single-cells. In addition, dual-asymmetric PCR primers were designed to identify the bacteria and reveal, when present, the ARG carriers. Finally, amplicon-based sequencing was used to sequence fused product of gene pairs and generate single-cell profile of the heterogeneous laboratory sample.

Results: The method described here offers new insights on how to apply ddPCR system to track the identity of functional gene carriers in heterogeneous samples and is a promising tool for microbial ecology and antibiotic resistance research.

PT380 The role of LytR-CpsA-Psr (LCP) proteins in Mycobacterium smegmatis

Abhipsa Sahu[1], Ziwen Xie[1], Siauw Liang Liem[2], Roy Ummels[2], Wilbert Bitter[2], David Ruiz-Carrillo[1], Boris Tefsen[1]

[1]Xi'an Jiaotong-Liverpool University, Department of Biological Sciences, Suzhou, China
[2]VU University Medical Center, Department of Medical Microbiology and Infection Prevention, Amsterdam, Netherlands

Background: Tuberculosis caused by *Mycobacterium tuberculosis*, is one of the leading causes of mortality worldwide. With an upsurge of multidrug-resistant tuberculosis and rifampicin-resistant tuberculosis, development of new drugs need immediate attention, and this needs identification of potential drug targets.

Objectives:

- To determine the enzymatic role of LCP proteins in *Mycobacterium smegmatis* (*Msmeg*).
- To study the physiological effects caused by single and double deletion LCP mutants.

Methods:

- Determination of pyrophosphatase activity by measuring the inorganic phosphate released.
- Determination of physiological effects by growth kinetics, colony morphology, aggregation rate, biofilm formation, minimum inhibitory concentration, and oxidative stress by quantitative RT-PCR.

Results: Our study is focused on the enzymatic role of LCP homologs in *Msmeg*, viz., *MSMEG_0107*, *MSMEG_1824*, *MSMEG_5775* and the rather ignored *MSMEG_6421*, and the physiological effects caused by single and double deletion LCP mutants. We found *MSMEG_0107* and *MSMEG_1824* displaying maximum pyrophosphatase activity similar to other studies. While single deletion mutants of all LCP genes except *MSMEG_1824*, which is essential, showed no or moderately altered phenotypes, the cell envelope of the double-deletion LCP mutant, *ΔΔ(0107+5775)* was severely compromised. This strain has a deprived growth rate, slower aggregation, diminished biofilm formation on air-liquid interface, altered colony morphology and increased susceptibility to rifampicin and other antibiotics. Under oxidative stress, all LCP homologues are differentially regulated, as shown by quantitative RT-PCR. We hypothesize that *MSMEG_0107* and *MSMEG_5775* aid coupling of arabinogalactan to peptidoglycan in *Msmeg* in a complementary manner and, under specific conditions when *MSMEG_1824* alone cannot perform this function.

PT381 Structural investigation of Enterohemorrhagic Escherichia coli type IV pilus and pilins

Theis Jacobsen[1], Benjamin Barduaux[1], Nadia Izadi-Pruneyre[1], Michael Nilges[1]

[1]*Institut Pasteur, Paris, France*

Background: Bacterial type IVa pili (T4aP) are involved in adherence, twitching motility and molecular transport. These proteinaceous filaments attached to the surface of the bacteria are very flexible and are highly conserved in enterobacteria. The expression of T4aP in Enterohemorrhagic *Escherichia coli* (EHEC) facilitates adhesion to intestinal epithelia, causing intestinal infections, with hemolytic uremic syndrome (HUS) as a complication.

Objectives: The structures of pili and their assembly platform have been of great interest to clarify the mechanism of action for adherence. The molecular basis of the T4aP is the major pilin, named PpdD in EHEC, which constitutes the bulk of the proteins in the pilus.

Methods: An NMR structure of the periplasmic domain of PpdD, excluding the N-terminal transmembrane helix, has been determined (Bardiaux et al; 2019 in revision). To investigate the topology of whole pili, cryo-electron microscopy (cryo-EM) has been employed to obtain an EM density map of the assembled pili and dock the structure of the PpdD pilin.

Results: Four other pilins have been identified in EHEC. These are predicted to be minor pilins (PpdA, PpdB, ygdB and PpdC) and are present in much lower abundance compared to the major pilins. In the homologous type II secretion system (T2SS) pseudopili, it has been shown that the minor pilins form a complex, which is thought to initiate the formation of pili. Structural determination of the minor pilins and the complex will be crucial for targeted drug or vaccine development against EHEC.

PT382 Biosynthesis of cysteine and methionine from dimethylsulfoniopropionate in Ruegeria pomeroyii DSS-3

Joseph Wirth[1], Tao Wang[1], William Whitman[1]

[1]*University of Georgia, Department of Microbiology, Athens, United States*

Background: Dimethylsulfoniopropionate (DMSP) is abundant in marine environments and acts as an important source of reduced carbon and sulfur for marine bacteria. Previous research has shown that marine bacteria which produce methanethiol from DMSP also incorporate the DMSP sulfur into methionine. These results led to the hypothesis that methionine is synthesized from DMSP via the direct capture of methanethiol as a single unit. However, it is also possible that methionine is formed by the random reassembly of sulfide and methyl-tetrahydrofolate formed from DMSP.

Objectives: In this study, these alternatives were distinguished during growth of *Ruegeria pomeroyi* DSS-3 in a chemostat grown with 2 mM glucose and a mixture of 50 µM doubly-labeled DMSP [di(methyl-^{13}C)sulfonio-^{34}S-propionate] and 50 µM unlabeled DMSP (100 µM total DMSP).

Methods: Amino acids were extracted from hydrolyzed cellular proteins, and the cysteine and methionine were purified by TLC. Mass spectroscopy was then used to determine the labeling of each amino acid.

Results: Under these conditions, one-third of the methionine is synthesized via the direct capture of methanethiol and the remainder is synthesized via the random reassembly of sulfide and methyl-tetrahydrofolate. Moreover, DMSP is the major source of cellular sulfur even in the presence of a large excess of sulfate. In contrast, DMSP does not fully label the cellular methylene tetrahydofolate or methyl tetrahydrofolate pools, suggesting that much of the C-1 carbons are oxidized.

PT383 phylogenomic analysis of alcaligenes spp. indicates five genomic clusters, revealing a. faecalis subsp. phenolicus reclassification to a. phenolicus sp. nov.

Roberto E. Duran[1], Valentina Mendez[1], Eduardo Castro-Nallar[2], Edward Moore[3;4], Michael Seeger[1]

[1]Molecular Microbiology and Environmental Biotechnology Laboratory, Department of Chemistry, Valparaiso, Chile
[2]2 Centro de Bioinformática y Biología Integrativa, Facultad de Ciencias Biológicas, Universidad Andrés Bello, Santiago, Chile
[3]Department of Infectious Diseases, Institute for Biomedicine, Sahlgrenska Academy, University of Gothenburg, Culture Collection University of Gothenburg (CCUG), Sahlgrenska Academy , Gothenburg, Sweden
[4]Centre for Antibiotic Resistance Research (CARe), University of Gothenburg, Gothenburg, Sweden

Background: *Alcaligenes* genus is currently comprised of four species, based on genotypic and phenotypic characteristics: *A. aquatilis*; *A. endophyticus*; *A. faecalis*; and *A. pakistanensis*. Genomes corresponding to the *Alcaligenes* genus are predominantly represented by *A. faecalis* strains. Average nucleotide identity (ANI) analyses of genome sequences was introduced as a criterion for species identification. Analysis based on ANI have not yet been performed within the *Alcaligenes* genus.

Objectives: The aim of this study is to evaluate the taxonomic placement of 26 strains of *Alcaligenes* spp., using phylogenomic methods.

Methods: Genomes of strains of *Alcaligenes* spp. were obtained from GenBank (September 2018): 22 *A. faecalis,* 2 *A. aquatilis* and 2 *Alcaligenes* sp. genomes. For multi-locus sequence analysis (MLSA), 35 gene markers were retrieved from the genomic dataset. Nucleotide sequences were aligned, trimmed and concatenated. Bayesian Inference was used to calculate a distribution of probable trees as implemented in MrBayes. Pairwise alignments and all-against-all identities were estimated for the input genome sequences, using JspeciesWS.

Results: Five genomic clusters were identified by MLSA. The same pattern was observed, using ANI species threshold (clustering value >95%). Strains classified as *A. faecalis* were found in each cluster, indicating probable misclassifications within the genus. Cluster I contained the type strain for the *A. faecalis* species, while cluster II included the type strain for *A. faecalis* subspecies *phenolicus*. Cluster III included three strains classified as *A. faecalis*, as well as the two genomes of *A. aquatilis* species. Cluster IV and V contained only strains classified as *A. faecalis*.

PT384 Lactobacillus femina sp. nov., a new species of Lactobacillus delbrueckii group

Joana Rocha[1], João Francisco Tomaz Santos Botelho[1], Magdalena Ksiezarek[1], Teresa Goncalves Ribeiro[1], Miguel Paulo Machado[2], Joao Andre Carrico[2], Lígia Pimentel[3], Sofia Salsinha[3], Luís M. Rodríguez-Alcalá[3], Manuela Pintado[3], Svetlana Ugarcina Perovic[1], Luisa Maria Vieira Peixe[1]

[1]UCIBIO/REQUIMTE. Faculdade de Farmácia, Universidade do Porto
[2]Universidade de Lisboa, Faculdade de Medicina, Instituto de Microbiologia and Instituto de Medicina Molecular
[3]Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia

The *Lactobacillus* genus is a paraphyletic group of lactic acid bacteria. In the human body, lactobacilli colonize different niches playing important roles in preventing infection and maintaining health. Two strains, c10Ua161M^T and c10Ua232AE, were preliminary identified by MALDI-TOF-MS as *Lactobacillus jensenii*, but *pheS* gene phylogenetic analysis did not confirm this species attribution.

To define the taxonomic position of two strains isolated from urine sample of a reproductive-age healthy woman, using a polyphasic taxonomic approach.

Individual phylogenetic analysis of 16S rRNA and *pheS* genes, average nucleotide identity (ANI), genome-to-genome distance calculation (GGDC), cellular fatty acids and biochemical tests were performed for taxonomic classification.

Phylogenetic analysis based on 16S rRNA gene assigned both strains to the genus *Lactobacillus*. Additionally, *pheS*-based phylogenetic analysis revealed that c10Ua161M^T and c10Ua232AE shared >99% of similarity and formed a well discriminated cluster in the *Lactobacillus delbrueckii* group, with *L.jensenii* and *L.psittaci* type strains (92.6 and 84.6%, respectively) as the closely related species. However, ANI and GGDC values of these strains were clearly below the proposed criterion for bacterial species delineation (87.9<95% and 34.3<70%, respectively) when compared with the genome sequence of *Lactobacillus* type strains. The type strain c10Ua161M^T G+C content was 34.2 mol%. The main cellular fatty acid was oleic acid ($C_{18:1\,\omega9}$). The ability to metabolize melibiose and starch further discriminated the two strains from other closely related *Lactobacillus* species. These results support the description of a novel species of the genus *Lactobacillus*, for which the name *Lactobacillus femina* sp. nov. is proposed (c10Ua161M^T).

PT385 Insights into the phylogeny of false-branching heterocytous cyanobacteria with the description of Scytonema pachmarhiense sp. nov.

Aniket Saraf[1;2;3], Himanshu Dawda[1], Prashant Singh[2;3]

[1]*Ramniranjan Jhunjhunwala College, Department Of Botany, Mumbai, India*
[2]*National Centre For Cell Science राष्ट्रीय कोशिका विज्ञान केंद्र, National Centre for Microbial Resource, Pune, India*
[3]*Banaras Hindu University, Department Of Botany, Varanasi, India*

Background: The false branching heterocytous cyanobacteria are grouped under the families Scytonemataceae, Tolypothrichaceae, Godleyaceae and Rivulariaceae according to the current classification system. The recent phylogenetic studies have shown that the family Scytonemataceae along with its type genus *Scytonema* is polyphyletic. However, these reports are based upon few sequences and there has been no comprehensive phylogenetic studies performed in order to resolve the taxonomic complexities within the family Scytonemataceae. This has resulted into inconsistencies in the taxonomic status of the members of Scytonemataceae.

Objectives: To characterize freshwater cyanobacterium strain 10A1_PS using the polyphasic approach and decipher the phylogenetic complexity of false-branching heterocytous cyanobacteria.

Methods: The strain 10A1_PS was characterized by morphological analysis followed by molecular-phylogenetic analysis using 16S rRNA gene and 16S-23S ITS secondary structure analysis. The 16S rRNA gene tree was constructed using maximum number of false-branching heterocytous genera which was further used to deduce the taxonomic complexity.

Results: The polyphasic approach indicates that the strain 10A1_PS is a novel species of the genus *Scytonema* and is described as *Scytonema pachmarhiense*. Through our phylogenetic assessment, we observed that *Scytonema* is highly polyphyletic with novel pattern of clustering. This indicates that the clusters distant from *Scytonema sensu-stricto* demand revision. This study also provides preliminary evidence for the potential divergence within Scytonemataceae and emphasizes the need for description of more than one families from the existing Scytonemataceae. Further, 16S rRNA gene tree presented in this study can be used as a phylogenetic framework which will further contribute in resolving the taxonomic disputes.

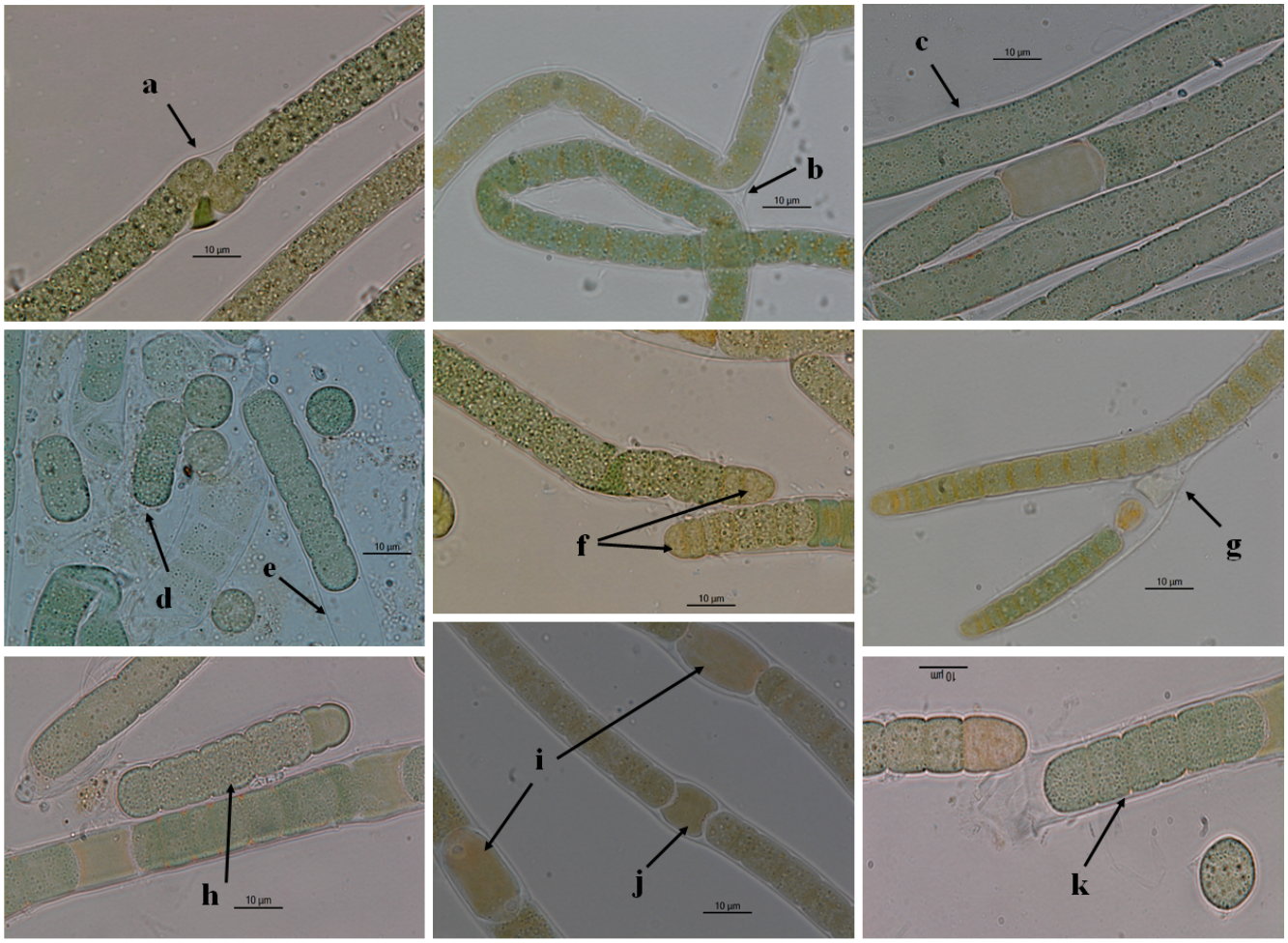


Fig 1: Morphological characteristics of ***Scytonema pachmarhiense*** (10A1_PS)
(a) Filament showing development of false branching; **(b)** Double false branching; **(c)** Old trichome having no constriction; **(d)** Hormogonia; **(e)** Newly developed hormogonia still inside the parent sheath; **(f)** Tapering ends of the terminal cells; **(g)** False branching; **(h)** Hormogonia with prominent constrictions and tapering terminal cells; **(i)** Heterocytes with convex and concave ends; **(j)** Separation disc; **(k)** Hormogonia inside the sheath having prominent constrictions

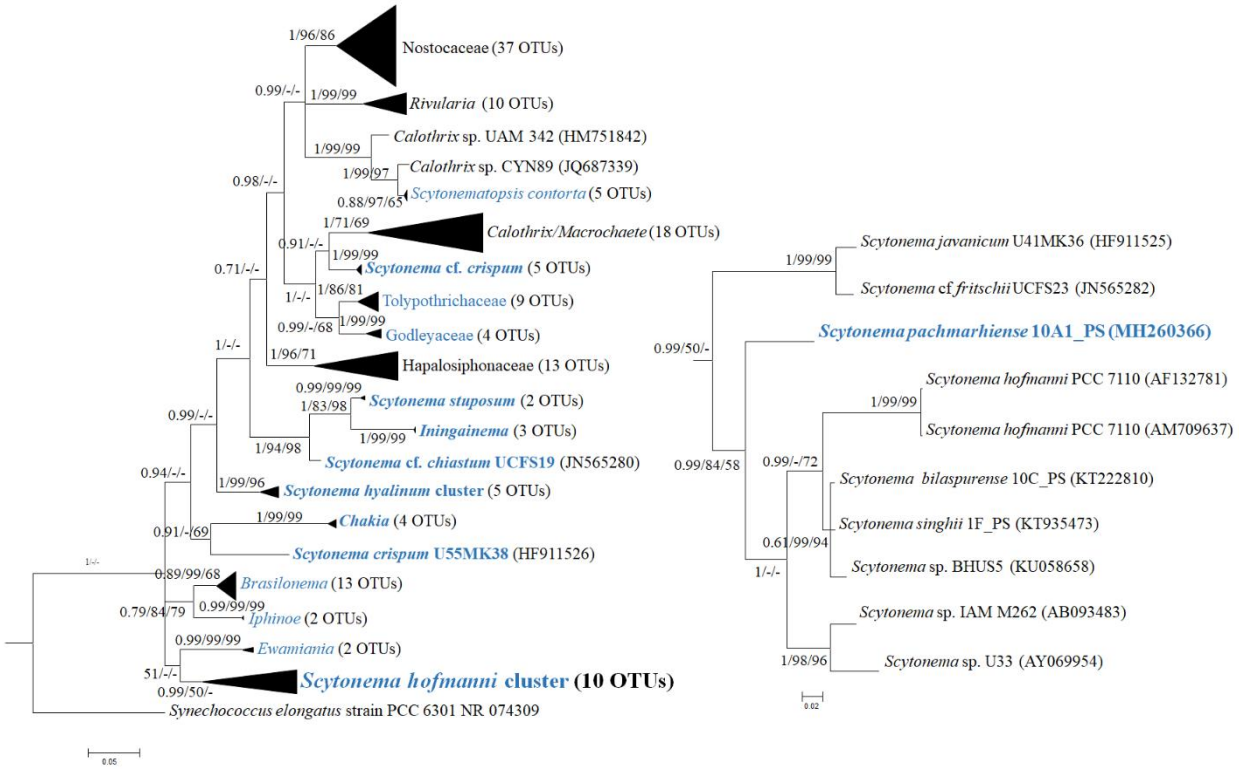
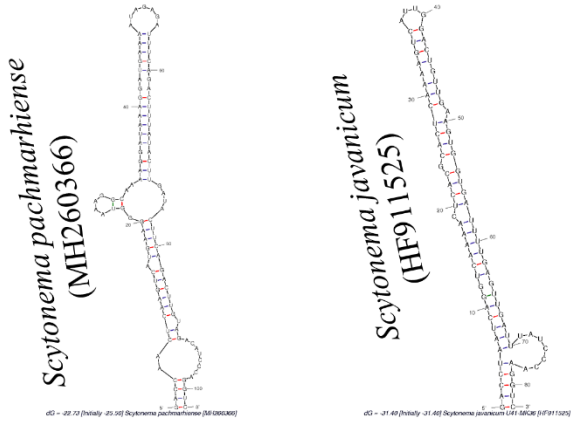


Fig 2: Phylogenetic positioning of the *Scytonema pachmarhiense* based on 16S rRNA gene inferred by Bayesian Inference tree with the probability scores/bootstrap values representing BI, NJ and MP respectively

D1-D1' helix region



boxB helix region

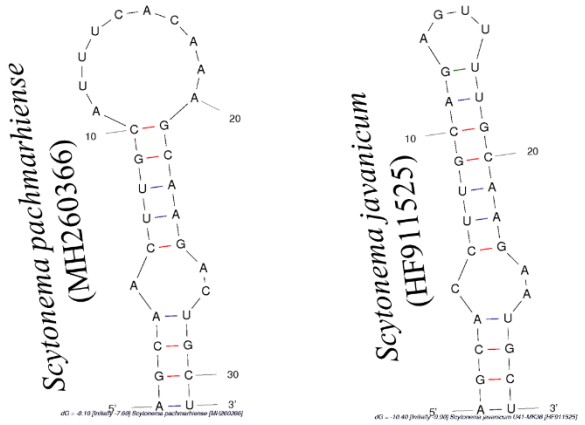


Fig 3: 16S-23S ITS folded secondary structures of D1-D1' and boxB helix region of *Scytonema pachmarhiense* and *Scytonema javanicum* U41MK36.

PT386 Genome-based bacterial identification system improves the taxonomic identities of the genome sequence in the public databases

Sung-min Ha[1], JE HEE LEE[1], Byung-Yong Kim[1], Youngwook Kim[1], Seokhwan Yoon[1], Jongsik Chun[1]

[1]ChunLab, Inc., Seoul, Korea, Rep. of South

Background: Correct identification of bacteria is crucial because, oftentimes, they are stored and used as a reference point. To date, there are numerous bacterial identification methods available including biochemical kits, MALDI-TOF mass spectrometry, and 16S rRNA. Because bacterial species is now defined by genome sequence comparisons, genome-based method, in theory, can identify unknown bacterium with almost perfect accuracy.

Objectives: To evaluate the genome-based identification system using genomes from the NCBI genome database.

Methods: A total of 121,101 genomes were used for this study. The species or subspecies-level identification was performed using the TrueBac ID system (https://www.truebacid.com). The results were compared with the species names given in the public databases.

Results: Out of 121,101 genomes in the NCBI database, TrueBac ID identified 115,179 (95.1%). The remaining genomes were not identifiable to species level due to the lack of type strain genomes. Among the identified genomes, 74,791 (64.9%) showed identical taxonomic names to that of NCBI. On the other hand, 11,957 (10.4%) and 19,031 (16.5%) genomes have identified further down into species and subspecies level, respectively. The rest of 9,400 (8.2%) genomes were considered misidentified because of their diffrences between given and identified species name. These discrepencies may be due to the lack of reference genome and different identification methods before genome sequencing was prevalent. The test here reiterates the importance of correct identification system. Finding a true identity of bacteria will benefit not only the research community but also the general public, who gets diagnosis and treatments based on these results.

PT387 Pseudomonas catumbelensis sp. nov. is a novel species within the Pseudomonas putida phylogenetic group isolated from a septic tank in Angola and carrying several antimicrobial resistance genes

João Francisco Tomaz Santos Botelho[1], Joana Rocha[1], Cédric Lood[2;3], João A. Carriço[4], Teresa Goncalves Ribeiro[1], Vera van Noort[2;5], Rob Lavigne[3], Filipa Grosso[1], Luisa Maria Vieira Peixe[1]

[1]*UCIBIO/REQUIMTE, FFUP, Microbiology, Porto, Portugal*
[2]*Centre of Microbial and Plant Genetics, KU Leuven, Department of Microbial and Molecular Systems, Leuven, Belgium*
[3]*Laboratory of Gene Technology, KU Leuven, Department of Biosystems, Leuven, Belgium*
[4]*Instituto de Microbiologia, Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Lisboa, Portugal*
[5]*Institute of Biology, Leiden University, Leiden, Netherlands*

The *Pseudomonas putida* phylogenetic group is composed of several opportunistic pathogens and environmental species. Previous studies addressing the phylogeny within the *Pseudomonas* genus have urged the need for sequencing the genome of type strains and correct species assignment to provide more robust phylogenomics.

Here, we conducted genotypic and phenotypic analyses to define the taxonomy and the pool of antimicrobial resistance genes present on a *Pseudomonas* spp. strain recovered from a septic tank in Catumbela, Angola.

MinION and Illumina reads were combined to fully assemble the genome. Multilocus sequence analysis (MLSA), average nucleotide identity (ANI), genome-to-genome distance calculation (GGDC) and biochemical tests were conducted for taxonomic classification. Annotation and exploring the genetic context of antimicrobial resistance genes were performed by Prokka and Galileo AMR, respectively.

The FFUP_PS_473^T strain was Gram-stain-negative, rod-shaped, motile and growth at 4-37ºC. The GC content of the FFUP_PS_473^T strain was 59.8%. Phylogenetic analyses based on MLSA assigned the strain to the *P. putida* group. ANI and GGDC values below the cutoff for species delineation (<95% and <70%, respectively) when compared with all sequenced *Pseudomonas* type strains confirmed that FFUP_PS_473^T belongs to a novel species within the *P. putida* group. This strain carries a new class 1 integron (named In1631 according to INTEGRALL) with the |*gcu189*|*cmlA8*|*bla*$_{OXA-246}$|*qnrVC1*|*arr-2*| cassette array encoding resistance to phenicol, beta-lactams, fluoroquinolones and rifampicin. In1631 is located within a Tn*402*-like defective transposon. Biochemical characteristics further differentiated FFUP_PS_473^T from other species of the genus *Pseudomonas,* for which the name *Pseudomonas catumbelensis* sp. nov. (FFUP_PS_473^T) is proposed.

PT388 Characterization of strains isolated from root nodules of common bean in Greece

Evdoxia Efstathiadou[1], Dimitrios Savvas[1], Anastasia Tampakaki[1]

[1]*Agricultural University of Athens, Department of Crop Science, Athina, Greece*

Background: *Phaseolus vulgaris* (L.), commonly known as bean or common bean, is a legume species that is cultivated worldwide as a grain or vegetable crop, and is a promiscuous host that forms root nodules with a wide range of symbiotic nitrogen-fixing bacteria belonging to the genera *Rhizobium*, *Ensifer* and *Burkholderia*. This is the first report on the characterization of bean-nodulating rhizobia in Greece.

Objectives: The goals of this research were (i) to isolate and characterize rhizobia nodulating local common bean genotypes grown in six different edaphoclimatic regions of Greece; and (ii) to assess their competitiveness and tolerance to environmentally stressful conditions.

Methods: Phenotypic, biochemical and molecular approaches were used to estimate the diversity of bacteria within nodules collected from field-grown common bean genotypes. The genetic diversity of the rhizobial isolates was assessed by BOX-PCR and the phylogenetic affiliation was assessed by multilocus sequence analysis (MLSA) of housekeeping and symbiotic genes.

Results: A total of 133 fast-growing rhizobial strains were isolated and grouped into 15 clusters by BOX-PCR. MLSA of representative isolates of each cluster revealed that some of them were affiliated to defined *Rhizobium* species whereas others might represent novel genomic lineages. Interestingly, a high occurence of isolates belonging to the *Caballeronia* genus were found within bean nodules. Although *Caballeronia* species have been isolated from root nodules, there is no evidence that any of the defined *Caballeronia* species is able to nodulate legumes. The phylogenetic affiliation of the *Caballeronia*-like isolates and their ability to nodulate common bean is under investigation.

PT389 Comparison of the cell morphological properties examined with whole-mount ice-embedded cryo transmission electron microscopy between 5 genera in family Mycobacteriacea.

Hiroyuki Yamada[1], Kinuyo Chikamatsu[1], Akio Aono[1], Kazuyoshi Murata[2], Naoyuki Miyazaki[2;3], Yoko Kayama[4], Nagatoshi Fujiwara[5], Shinji Maeda[6], Satoshi Mitarai[1;7]

[1]The Research Institute of Tuberculosis, Japan Anti-Tuberculosis Association, Department of Mycobacterium Reference and Research, Kiyose, Tokyo, Japan
[2]National Institute of Physiological Science, Okazaki, Aichi, Japan
[3]Osaka University, Osaka, Japan
[4]Terabase, Okazaki, Aichi, Japan
[5]Tezukayama University, Nara, , Japan
[6]Hokkaido Pharmaceutical University, Sapporo, Hokkaido, Japan
[7]Nagasaki University, Nagasaki, Japan

Background: Former genus *Mycobacterium* has been divided into emended genus *Mycobacterium* and four novel genera, *Mycobacteroides*, *Mycolicibacterium*, *Mycolicibacter* and *Mycolicibacillus*.

Objectives: Comparisons of the fundamental morphological properties of bacterial cells of 24 species belonging to the 5 genera was performed with whole-mount ice-embedded cryo transmission electron microscope (cryo-TEM) examination.

Methods: *Mycobacterium*; 11 species, *Mycolicibacterium*; 7 species, *Mycobacteroides*, *Mycolicibacter* and *Mycolicibacillus*; each 2 species. Bacteria were cultured with Middlebrook 7H9 liquid medium, fixed with 2.5% glutaraldehyde and rinsed with phosphate buffer. Cells were quick-frozen and whole-mount ice-embedded samples were examined with cryo-TEM. Totally, more than 1,000 cells were examined and TEM images were analyzed with Fiji/ImageJ, where diameter, length, perimeter, circularity and aspect ratio of each cell were measured.

Results: Average cell diameter, length and perimeter of genus *Mycolicibacterium* (0.65 µm, 2.53 µm and 5.58 µm, respectively) were significantly longer than those of other 4 genera. Average diameter of genus *Mycobacterium* (0.55 µm) was significantly longer than that of genera *Mycolicibacter* and *Mycolicibacillus*. Average length of genus *Mycobacterium* (2.08 µm) was significantly longer than that of genera *Mycobacteroides*, *Mycolicibacter* and *Mycolicibacillus*. On the other hand, average cell circularity of genus *Mycolicibacillus* (0.82) was significantly higher than those of other 4 genera. These morphological data strongly support the division of the genus *Mycobacterium* into the five genera. Furthermore, because genera *Mycobacterium* and *Mycolicibacterium* are comprise of species with a variety of morphological properties, further division of these two genera into novel genera might be achieved in future.

PT390 Phylogenetic and genomic insights into the genus Citrobacter unveils two novel genera

Teresa Goncalves Ribeiro[1], Bruno Ribeiro Gonçalves[2], João André Carriço[2], Luísa Peixe[1]

[1]*UCIBIO/REQUIMTE. Faculty of Pharmacy, University of Porto, Portugal*
[2]*Universidade de Lisboa, Faculdade de Medicina, Instituto de Microbiologia and Instituto de Medicina Molecular, Portugal*

Background: Classification of *Citrobacter* species have been largely based on DNA-DNA hybridizations and/or 16S rRNA gene analyses. Their limited taxonomic resolution and the polyphyletic structure of *Citrobacter* sp. observed in 16S-rRNA gene-based phylogenetic trees motivated a reassessment of *Citrobacter* spp. classification.

Objectives: Clarification of the taxonomic standing of *Citrobacter* species employing comparative phylogenetic and genomic approaches.

Methods: Multilocus sequence analysis (MLSA), *recN*-based phylogenetic analysis, average nucleotide identity (ANI), and genome-to-genome distance calculation (GGDC) were conducted using genomes of *Citrobacter* spp. and species belonging to closely related genera (*Salmonella*, *Escherichia*, *Kluyvera* and *Klebsiella*) type strains. The core-genome schema was defined using chewBBACA on 195 publicly available *Citrobacter* sp. genomes. The resulting allelic profiles and profiles obtained from genomes of the closely related genera were compared using minimum spanning trees (MST).

Results: MLSA- and *recN*-phylogenetic tree clearly demonstrated that *Citrobacter* genus is not monophyletic, with *Citrobacter* species being grouped into two distinct clusters and one branch (*Citrobacter koseri*). ANI values between *Citrobacter* clusters (80.36-81.96%) and between each cluster and *Salmonella enterica* LT2^T (80.38-81.30% and 80.67-81.12%, respectively) were similar. GGDC values between *Citrobacter* clusters (23.90-25.50%) and between each cluster and *C.koseri* (26.00-26.90 %) were clearly below the cut-off level (70%) for species delineation. The MST analysis revealed highly diverse intra-species allelic profiles. Nevertheless, MST-clusters (cut-off at 830 of 895 loci) are congruent with the proposed classification using *recN*/MLSA. Therefore, species of cluster I should be assigned to *Citrobacter* sensu lato, while species from cluster II and *C.koseri* should be reclassified into novel genera.

PT391 Phylogenomic analyses exploring intra-generic heterogeneity within the biomedically and industrially important genus Amycolatopsis

Vartul Sangal[1], Michael Goodfellow[2], Hans-Peter Klenk[2], Iain Sutcliffe[1]

[1]Northumbria University, Applied Sciences, Newcastle upon Tyne, United Kingdom
[2]Newcastle University, School of Natural and Environmental Sciences, Newcastle upon Tyne, United Kingdom

Background: Prokaryotic systematics provides the foundation for microbial research of ecological, industrial and medical importance. Traditional polyphasic approaches used to define species tend to be time and labour-intensive, problems compounded by a lack of reproducibility and/or low resolution. Recent genome-based studies highlight widespread misclassification and misidentification of strains using these approaches. Therefore, we have extended the phylogenomic approaches to the industrially important genus *Amycolatopsis* that encompasses strains capable of producing novel bioactive molecules, notably new antibiotics.

Objectives: To investigate the extent of misclassification and misidentification of *Amycolatopsis* and to resolve the intra-generic structure of the genus.

Methods: Genomes of five *Amycolatopsis* strains were sequenced and analysed using phylogenomic and taxogenomic approaches along with 52 other publically available genomes and 12 representatives from related genera.

Results: The genus *Amycolatopsis* is comprised of four major clades. However, three strains grouped outwith the genus. *Amycolatopsis halophila* YIM93223^T is phylogenetically and taxonomically distinct from main *Amycolatopsis* clade and belongs to a distinct genus. In turn, *Amycolatopsis palatopharyngis* DSM 44832^T and *Amycolatopsis marina* CGMCC4 3568^T formed a clade close to *Saccharomonospora* strains though associated taxogenomic matrices indicate that they may merit generic status. In addition, four strains have been given the wrong species designation and three unclassified *Amycolatopsis* spp. strains can be assigned to known species, thereby providing further evidence that classification based on whole genome sequence data can reliably resolve intra- and inter-generic relationships within prokaryotic genera.

PT392 Polyphasic characterization of Flavobacterium sp. GS03, a protease-producing bacterium isolated from sediment of the Nackdong River

Hyangmi Kim[1]

[1]Nakdonggang National Institute of Biological Resources(NNIBR), Bioresources Culture Collection Division, Sangju-si, Gyeongsangbuk-do, Korea, Rep. of South

Background: A protease-producing bacterium GS03^T isolated from sediment of the Nackdong River in sangju, Korea, needs accurate taxonomic position.

Objectives: The aim of the present study was to describe the isolation and polyphasic characterization of strain GS03^T, as representing a novel species of the genus *Flavobacterium*.

Methods: Genomic DNA was extracted using the DNeasy Blood and Tissue kit. Fatty acid methyl esters were obtained from fresh wet biomass, separated, identified and quantified with the Sherlock microbial Identification System. Quinone was extracted with chloroform/methanol (2:1, v/v) and analyzed by HPLC.

Results: Cells were Gram-stain-negative, aerobic, rod-shaped and positive for catalase and oxidase. Optimal growth occurred at 10–30 °C (optimum 25 °C), at pH 7.0–8.5 (optimum pH 7.5), and at an optimum NaCl concentration of 0 % (w/v). The major cellular fatty acids (>10 % of the total) were iso $C_{15:0}$, iso $C_{15:1}$ G, $C_{15:1}$ *ω6c,* iso $C_{15:0}$ 3-OH and iso $C_{17:0}$ 3-OH. The major respiratory quinone was menaquinone MK-6. The whole genome of GS03 was 1,8 Mb with G+C content of 37.9 mol%. The major polar lipids of the isolate were, phosphatidylethanolamine three unidentified aminolipids, two unidentified phospholipids, one unidentified lipid and one unidentified aminophospholipid. Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain GS03^T clustered with *Flavobacterium paronense* KNUS1T^T (96.8 %) within the genus *Flavobacterium*. Based on the polyphasic characterization, strain GS03^T represents a novel species of the genus *Flavobacterium*, for which the name *Flavobacterium sangjuense* sp. nov. is proposed.

PT393 Phylogenomic characterisation of novel Corynebacterium isolates associated with fatal diphtheritic stomatitis in yellow-eyed penguins in New Zealand

Sarah Saunderson[1], Anne Midwinter[2], Melanie Young[1], Kate McInnes[3], Jim Watts[4], Vartul Sangal[5]

[1]*University of Otago, Department of Microbiology and Immunology, Dunedin, New Zealand*
[2]*Massey University, School of Veterinary Science, Palmerston North, New Zealand*
[3]*Department of Conservation, Science and Capability, Wellington, New Zealand*
[4]*Department of Conservation, Coastal Otago District Office, Dunedin, New Zealand*
[5]*Northumbria University, Applied Sciences, Newcastle upon Tyne, United Kingdom*

Background: Yellow-eyed penguins, *Megadyptes antipodes*, are endangered with the numbers of breeding pairs rapidly declining. Diphtheritic stomatitis is one of the major causes of high mortality in mainland populations, especially among young chicks. *Corynebacterium* isolates of undefined species have been associated with the diphtheritic lesions but the mechanism of pathogenesis is unknown.

Objectives: This study attempts to infer virulence mechanisms and to identify effective vaccine candidates against these *Corynebacterium* strains.

Methods: Whole genomes of 16 *Corynebacterium* strains isolated from the lesions or the oral cavities of yellow-eyed penguins were sequenced and analysed using a number of comparative genomic, phylogenetic and taxogenomic approaches.

Results: These analyses revealed that the *Corynebacterium* isolates belong to a novel species, *Corynebacterium hoiho* sp. nov. and were subdivided into two distinct lineages. Lineage-1 was relatively homogenous and more prevalent among yellow-eyed penguin chicks from different sampling sites than Lineage-2. Genomic islands introduced significant diversity among these strains with minor functional variations between the two lineages. The genes encoding SpaDEF type surface pili which may play key role in adhesion to cells in the oral cavities are present among all the strains. Also present are the virulence genes encoding cell wall-associated hydrolase (CwlH) and peptidoglycan endopeptidase (RipA) that are involved in cell surface organisation and may also assist in adhesion to the host cells. Interestingly, these strains possess a gene encoding phospholipase D, an exotoxin responsible for intracellular persistence and spread of pathogenic bacteria. A vaccination with inactivated phospholipase D may protect yellow-eyed penguins from 'diphtheritic stomatitis'.

PT394 A novel moderately thermophilic, anaerobic saccharolutic bacterium isolated from a Kamchatka hot spring

Galina Slobodkina[1], Elizaveta Bonch-Osmolovskaya[1], Alexander Slobodkin[1]

[1]*Winogradsky Institute of Microbiology, Research Center of Biotechnology of the Russian Academy of Sciences*

Background: Uzon Caldera, the largest geothermal area in the Kamchatka Peninsula (Russia), hosts the numerous hot springs with a significant input of organic matter derived from the higher vegetation. The initial steps of degradation of plant residues in these anoxic habitats are performed by thermophilic carbohydrate-utilizing microorganisms. Thermophiles have shown remarkable promise in terms of their applications in modern biotechnology.

Objectives: The aim of the study was a search for new saccharolytic, hydrogen-producing, anaerobic, thermophilic microorganisms from hydrothermal ecosystems.

Methods: Methods of isolation and phenotypic and genotypic characterization to fulfill the requirements for the description of the new taxa were used.

Results: A novel moderately thermophilic, anaerobic bacterium, strain STR9^T was isolated and characterized. Cells of strain STR9^T were spore-forming motile straight or slightly curved rods having a Gram-positive cell-wall structure. The temperature range for growth was 30–58°C, with an optimum at 50°C. The pH range for growth was 5.0–10.5, with an optimum at pH 8.0–9.0. NaCl inhibited growth of the strain STR9^T and no growth was evident above 0.5% NaCl (w/v). Several mono-, di-, and polysaccharides as well as beef extract and yeast extract supported growth. The main end products of glucose fermentation were ethanol, acetate, hydrogen, and carbon dioxide. On the basis of morphological, physiological and ecological properties and the low sequence similarity of 16SrRNA gene with related taxa, we propose strain STR9^T to represent a novel species of a new genus, *Tepidibaculum saccharolyticum* gen. nov., sp. nov. within the family *Ruminococcaceae*, the order *Clostridiales.*

PT395 Genetic marker gene, recQ, differentiating Bacillus subtilis and the closely related species

Jun Heo[1], Soo-Jin Kim[1], Jeong-Seon Kim[1], Seung-Beom Hong[1], Byeong-Yong Park[1], Soon-Wo Kwon[1]

[1]*National Institute of Agricultural Sciences, Agricultural Microbiology Division, Wanju, Korea, Rep. of South*

Background: Taxonomic classification and exact identification of *Bacillus subtilis* and related species are important.

Objectives: The aim of this study is to clarify the taxonomy of *Bacillus subtilis* and related species.

Methods: *Bacillus subtilis* and related species taxonomy was conducted using *RecQ* which encodes a DNA helicase and genome sequences.

Results: Sequence similarity of *recQ* type strains among *Bacillus* species used in this study ranged from 66.2-96.6 %, whereas OrthANI ranged from 72.6-95.8 %. According to the phylogenetic tree based on *recQ* sequences, each type strain of all *Bacillus* species or subspecies used in this study was placed in a unique taxonomic position. Four *B. subtilis* subspecies, *B. tequilensis* and *B. vallismortis* were grouped in one compact cluster (Cluster A). Type strain of *B. subtilis* subsp. *subtilis* were classified into A1 cluster, and divided into subgroups. Isolates from Natto were classified into one subgroup, whereas those from Cheonggukjang were divided into several subgroups within A1. Differences in genetic diversity between strains from Cheonggukjang and Natto originate from the source and inoculation method of strains involved in the fermentation process. Type strains of *B. halotolerans* and *B. mojavensis* were grouped into one compact cluster (B), related to A. *B. siamensis*, *B. velezensis,* and *B. amyloliquefaciens* were grouped into an independent cluster (C). Sequencing of *recQ* were useful for identification of *Bacillus subtilis* and closely related species, which were inseparable via 16S rRNA sequence.

PT396 A novel subspecies of the Clostridial bacteria that exacerbate pathosis in a mouse model of ulcerative colitis

Yoshiaki Kawamura[1], Tohru Miyoshi-Akiyama[2], Koji Mori[3], Masahiro Hayashi[4], Junko Tomida[1], Yuji Morita[5], Kaori Tanaka[4], Yoshiaki Kawamura[1]

[1]*Laboratory of Microbiology, Aichi Gakuin University Graduate School of Pharmaceutical Science, Nagoya, Japan*
[2]*Pathogenic Microbe Laboratory, Research Institute, National Center for Global Health and Medicine, Tokyo, Japan*
[3]*Biological Resource Center, National Institute of Technology and Evaluation, Chiba, Japan*
[4]*Division of Anaerobe Research, Life Science Research Center, Gifu University, Gifu, Japan*
[5]*Department of Infection Control Science, Meiji Pharmaceutical University, Tokyo, Japan*

Background: Ulcerative colitis is a chronic enteritis characterized by symptoms such as recurrent bloody diarrhea and abdominal pain. Despite the number of patients is increasing worldwide, the underlying mechanisms are not understood completely, and no fundamental treatment method has been established. We discovered that specifically increased *Clostridium* sp. PAGU 1678 in feces of DSS administrated mouse model. Furthermore, it was demonstrated that colitis becomes exacerbate by orally administering this strain to the mouse model.

Objectives: To clarify the correct taxonomic position of PAGU 1678, a comparative taxonomic study using *P. bifermentans* PAGU 2008^T (=JCM 1386^T) and the closely related bacterial species was carried out.

Methods and Results: Phylogenetic analysis of PAGU 1678 based on the 16S rRNA gene showed high homology (99.9%) with *Paraclostridium bifermentans*. Despite the close similarity, DNA homology value based on *wet* and *in silico* hybridization between PAGU 1678 and *P. bifermentans* PAGU 2008^T was 60.03% and 96.17%, respectively. Biochemically, PAGU 1678 could be differentiated from *P. bifermentans* PAGU 2008^T by H_2S production. Furthermore, PAGU 1678 was characterized by the presence of two phospholipids with different polarity on polar lipid analysis. In addition, PAGU 1678 was different from *P. bifermentans* PAGU 2008^T based on whole-cell protein analysis and MALDI-TOF MS.

On the basis of these biochemical and genetic characteristics lead us to conclude that strain PAGU 1678 should be categorized as a novel subspecies. This strain was officially recognized as *Paraclostridium bifermentans* subsp. *muricolitidis* subsp. nov. with PAGU 1678^T (=CCUG 72489^T =NBRC 113386^T) as the type strain.

PT397 Genetic diversity of indigenous rhizobia nodulating Vicia faba in Greece

Evdoxia Efstathiadou[1], Dimitrios Savvas[1], Anastasia Tampakaki[1]

[1]*Agricultural University of Athens, Department of Crop Science, Athina, Greece*

Background: *Vicia faba* L. is an important leguminous crop cultivated worldwide for food and feed. Considering the European guidelines towards sustainable agriculture, and the agro-economic importance of *V. faba*, the selection and the application of efficient rhizobial inoculants is a crucial lever for increasing legume crop yields. Despite that *Rhizobium leguminosarum* sv. *viciae* (*Rlv*) is the most common symbiont of *V. faba* worldwide, genetic differences in specificity among strains of *Rlv* have been reported. Other rhizobial species have also been reported as microsymbionts of faba beans.

Objectives: Considering the lack of systematic studies of rhizobia nodulating *V. faba* in Greece, this study aimed to reveal the genetic diversity and phylogeny of indigenous rhizobia nodulating diverse varieties of faba beans grown at different locations in Greece.

Methods: Phenotypic, biochemical and molecular approaches were used to estimate the diversity of rhizobia nodulating various field-grown faba bean genotypes. The genetic diversity of the rhizobial isolates was assessed by applying the BOX-PCR fingerprinting technique and the phylogenetic affiliation was assessed by multilocus sequence analysis (MLSA) of housekeeping and symbiotic genes.

Results: A total of 273 rhizobial strains isolated from faba bean nodules were analyzed by BOX-PCR and were divided into 30 clusters. Representative isolates of each cluster were further analyzed. Most of the isolates were effective microsymbionts of *V. faba* and were classified into *Rlv* group based on the phylogeny of housekeeping and symbiotic genes. However, some strains revealed genetic differences in housekeeping and symbiotic genes indicating that they might represent novel genomic lineages.

PT398 The International Code of Nomenclature of Prokaryotes, coping with the names of existing and novel taxa - "if it ain't broke don't fix it?"

Brian J. Tindall[1]

[1]*Leibniz-Institut DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH., Braunschweig, Germany*

The International Code of Nomenclature of Prokaryotes (2008 revision, the Code) that is based on changes made to the 1975 and 1990 revisions of the Code that defines the way names are formed and used as part of prokaryote taxonomy.

There are, however, numerous problems that can be encountered in the literature.

One of the major issues is the failure of many working with prokaryotes to even be aware of the existence of the Code, let alone having an appreciation of the purpose or function of the Code. Other problems include incorrect reference to the Code including incorrect citation or interpretation. Finally, problems have been introduced into the Code that either make implementation of the Code difficult or indicate that fundamental errors were made during the writing of the 1975 revision.

In order to fully appreciate the extent of the problem it is necessary to break down the Code into the fundamental principles, analyse their function and the way the dependent parts interact. A key aspect in appreciating where problems arise is the alternative interpretation of the same text or the publication of interpretations that seem to be far removed from the published wording. Only when these problems can be fully documented is it possible to make sure that the Code can be re-written and begin to fulfil its original intention of maintaining stability in prokaryote nomenclature and also forming the cornerstone of the way names are formed and subsequently used.

PT399 On the return of phenetics and numerical taxonomy?

Brian J. Tindall[1]

[1]*Leibniz-Institut DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH., Braunschweig, Germany*

Phenetics was coined by Cain and Harrison (1963) for a concept in classification: “we shall refer to the arrangement by overall similarity, based on all available characters with-out any weighting as phenetic, since it employs all observable characters (including, of course, genetic data when available)”. Sneath was one of the pioneers of numerical taxonomy (ie the use of computers in classification) in microbiology (Sokal & Sneath, 1963; Sneath & Sokal, 1972) and although he followed a numerical phenetic approach “Numerical Taxonomy” did not exclude the rising area of cladistics, originally pioneered by Hennig and termed “Phylogenetic Systematics”.

Much water has now passed under the bridge and existing terminology has been redefined (ie phylogenetic systematics) or new terms been coined such as phylogenomics.

However, some of the basic elements remain and authors are not always aware of some fundamental principles or errors in interpretation. There is a common misinterpretation of the term “phenetics” to mean only phenotypic data or that numerical taxonomy is limited to the evaluation of only phentotypic data. With the increasing use of computers to evaluate data sets there is an accompanying reliance on computer based evaluation (ie numerical taxonomy). In addition the use of similarity values to either generate graphical representations of the data (phylogenetic trees) or to rely on overall similarity values, set thresholds/cutoffs or define a minimum number of genes to define taxa there is an underlying trend to use phenetic principles even if they are labelled as being phylogenetic.

PT400 On the taxonomy of the genus Corynebacterium Lehmann and Neumann 1896

Brian J. Tindall[1]

[1]*Leibniz-Institut DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH., Braunschweig, Germany*

The genus *Corynebacterium* was first created by Lehmann and Neumann (1896). Over the past decade there have been significant changes in the classification of species assigned to this genus.

The task of the present study was to examine the current classification of the genus in order to determine whether a revision of the classification is needed.

An examination of data published in the literature indicates that a wide range of parameters have been taken into consideration when characterising strains assigned to species within the genus C*orynebacterium*. This includes physiological/biochemical tests, chemical analysis of cell wall components together with more recent information gained from individual gene or whole genome sequences. An evaluation of the published data and the published interpretation provides a basis for re-examining the classification of this genus.

One of the key aspects in the classification of the genus *Corynebacterium* is that with the implementation of the Approved Lists of Bacterial Names in 1980 it was a poorly defined group of species. This continued well into the late 1990s where many species were added without due consideration being given to a “sound” definition of the genus. This has now spilled over into molecular work where the evaluation of the data is primarily based on accepting the genus name associated with the species and ignoring heterogeneity within the range of properties found within the group. In addition errors may be found in the literature having a significant effect on the way the nomenclature is interpreted. A revision is badly needed.

PT401 Adaptation to ecological niches through genomic specificities in genus Prevotela

Tomaž Accetto[1], Gorazd Avguštin[1]

[1]*University of Ljubljana, Biotechnical faculty, Animal Science department, Domžale, Slovenia*

Background: various species of the bacterial genus *Prevotella* are found along the mammalian gut starting at the oral cavity and rising dramatically (in abundance) in parts of the gut dedicated to plant biomass degradation (rumen and hindgut), which has been shown both by culture and by molecular approaches. The ecological function of prevotellas has been postulated to lie in soluble fibre degradation, but the solid evidence is scarce and the *Prevotella* diversity, though obvious, is currently not truly appreciated.

Objectives: To obtain novel insight into ecological/functional diversity of prevotellas through genomic analysis.

Methods: a collection of several novel *Prevotella* strains retrieved in our laboratory and through Hungate 1000 project was used and their genomic sequences were analysed using bioinformatic approach. The growth experiments were used to corroborate the bioinformatic predictions.

Results: we show by genomic analysis that the analysed rumen/hindgut *Prevotella* species (of which 16 are novel) possess extensive repertoires of polysaccharide utilization loci (PULs) and carbohydrate active enzymes targeting various plant polysaccharides and can thus be separated into generalists and specialists species, both in range of substrates targeted and in PUL combinations targeting the same broad substrate classes. The growth experiments corroborated the bioinformatic predictions supporting the nutrient niche partitioning as a major factor in cohabitation of *Prevotella* species. There is further evidence that a link between translation initiation specificities and bacterial lifestyle exists in *Prevotella* indicating that the adaptation to ecological niches at the genomic level extends beyond mere gene content.

PT402 Characterization of potentially novel Brucella spp.

Angelo Cartes[1], Nicole Zambrano[1], Victor Campos[1], Roberto Vidal[2], Angel Oñate[1]

[1]*University of Concepcion, Faculty of Biological Sciences, Concepcion, Chile*
[2]*University of Chile, Faculty of Medicine, Santiago, Chile*

Background: Brucellosis is a zoonosis of worldwide prevalence, being one of its causal agents *Brucella abortus*, which causes economic losses and health problems in humans. A study conducted in 2011 in Chile, analyzed 71 isolates of *B. abortus* of fetal remains for cattle abortion. A study was made of the variable number of repetitions in tandem with 16 loci, where one of the strains showed a similarity of less than 10% with the rest of the strains studied, called AO:01.

Objectives: The atypical strain AO-01 was characterized to evaluate if this strain would correspond to a new undescribed species of the genus.

Methods: The strain was phenotypically analyzed by microbiological traits compared to *B. abortus* 2308 and RB51 strains and their genotype was analyzed by DNA-DNA hybridization, 16S rRNA gene sequencing, and phylogenetic trees were created from MLVA-16 of ITS 16-23S region and genes recA, rpoD, fusA and gyrB.

Results: The results show a different phenotype of the AO-01 strain compared to six Brucella species, due to its rapid growth, glucose fermentation, antimicrobial resistance and biofilm formation. The genomic analyzes showed a percentage of similarity lower than 64.8% in the DNA-DNA hybridization test, when compared with B. abortus 2308. When performing the taxonomic assignment of AO-01 through phylogenetic affiliation using the maximum likelihood algorithm, a distant classification was obtained compared to the *Brucella* species. Therefore, it is suggested that the isolate may represent a new undescribed species of the genus *Brucella*. Supported by Grants Fondecyt 1180122 and Fondecyt 1161161.

PT403 Whole genome sequencing of a novel Chryseobacterium strain isolated from poultry feather waste

Adeline Lum Nde[1], George Charimba[2], Arina Hitzeroth[3], Lize Oosthuizen[1], Laurinda Steyn[1], Jeff Newman[1;4], Celia Hugo[1]

[1]*University of the Free State, Microbial, Biochemical and Food Biotechnology, Bloemfontein, South Africa*
[2]*Cape Peninsula University of Technology, Department of Food Technology, Bellville, South Africa*
[3]*University of the Western Cape, Department of Biotechnology, Bellville, South Africa*
[4]*Lycoming College, Biology Department, Williamsport, United States*

Background: Genotypic methods (16S rRNA, Whole-genome sequencing, DNA-DNA hybridization, Average Nucleotide Identity and Average Amino Acid Identity) are nowadays included in prokaryotic delineation to take into account the overall genome relatedness of microorganisms.

Objectives: To use whole-genome sequencing to indicate a new species of *Chryseobacterium* isolated from chicken feather waste.

Methods: Genomic DNA of *Chryseobacterium* sp. 1_F178^T was extracted with a NucleoSpin® microbial DNA extraction kit (Macherey-Nagel) with the DNA quality checked with a Nanodrop ND-1000 (v3.3.0) spectrophotometer. The Nextera® XT DNA Library Prep kit was used to sequence the gDNA according to manufacturer's instructions. An Illumina MiSeq sequencer was used to sequence the genome and the assembly was performed with PATRIC database (https://www.patricbrc.org/), with SPAdes 3.10.0 as the assembly method. The sequenced genomes were uploaded to the Rapid Annotation with Subsystems Technology database (http://rast.nmpdr.org) for annotation.

Results: The two closest relatives of strain 1_F178^T as determined by 16S rRNA phylogenetic studies were *C. jejuense* and *C. nakagawai*. Both had 16S rRNA sequence similarity values (99.10 & 98.75 %) above the threshold value (98.5 %). Their DDH (31.4 & 32.7), ANI (86.4 & 86.6) and AAI (89.3 & 89.6) values were all less than the threshold value for species delineation. Strain 1_F178^T had 2982 genes in common with its closest relatives and 1094 unique genes. The 16S rRNA, DDH, ANI and AAI values were not within the threshold range for species delineation hence confirming strain 1_F178^T as a novel species of *Chryseobacterium*.

PT404 Chryseobacterium pennipullorum sp. nov., isolated from poultry feather waste

Lize Oosthuizen[1], George Charimba[2], Arina Hitzeroth[3], Adeline Lum Nde[1], Laurinda Steyn[1], Jeff Newman[1;4], Celia Hugo[1]

[1]*University of the Free State, Microbial, Biochemical and Food Biotechnology, Bloemfontein, South Africa*
[2]*Cape Peninsula University of Technology, Department of Food Technology, Bellville, South Africa*
[3]*University of the Western Cape, Department of Biotechnology, Bellville, South Africa*
[4]*Lycoming College, Biology Department, Williamsport, United States*

Background: Members of the genus *Chryseobacterium* have been isolated from environments such as terrestrial, aquatic, diseased animals, humans and food and some species may play a significant role in food spoilage. Food sources where *Chryseobacterium* species were found include poultry, red meat, milk and fish.

Objectives: To use whole genome sequencing data to describe a novel species in the genus *Chryseobacterium*.

Methods: The strain 7_F195^T was previously isolated from chicken feather waste collected from an abattoir in Bloemfontein, South Africa. Whole genome sequencing was performed to determine if strain 7_F195^T belongs to the genus *Chryseobacterium* and if the organism can be classified as a new species.

Results: The nearest neighbours based on 16S rRNA sequence similarity values (indicated in parentheses), were *C. flavum* KCTC 12877^T (98.42%), *C. indologenes* NBRC 14944^T (98.24%) and *C. gleum* ATCC 35910^T (97.71%). Genome sequencing revealed a genome size of 4,796,535 bp and a DNA G+C content of 38.6 mol%. The ANI values of strain 7_F195^T compared to *C. flavum, C. indologenes* and *C. gleum* were 81.45, 81.86 and 82.38%, respectively. The digital DNA-DNA hybridization values for strain 7_F195^T with *C. flavum*, *C. indologenes* and *C. gleum* were 23.7, 23.7 and 24.9%, respectively. This data indicated that strain 7_F195^T represents a novel *Chryseobacterium* species. The name *Chryseobacterium pennipullorum* sp. nov. is proposed. The type strain is 7_F195^T (=LMG 30781^T=KCTC 62760^T).

PT405 Revision of Chryseobacterium classification: genus distribution guided by observed iscontinuities in Amino Acid Identity values, a possible consequence of major extinction events

Ainsley Nicholson[1], Christopher Gulvik[1], Anne, Whitney[1], Ben Humrighouse[1], Melissa Bell[1], Aaron Villarma[1], Mili Sheth[1], Dhwani Batra[1], Lori Rowe[1], Mark Burroughs[1], Peter Kaempfer[2], Celia Hugo[3], Jeffrey Newman[4], Barry Holmes[5], Jean François Bernardet[6], John McQuiston[1]

[1]Centers for Disease Control and Prevention, Atlanta, United States
[2]University of Giessen, fInstitut für Angewandte Mikrobiologie, Gießen, Germany
[3]University of the Free State, Department of Microbial, Biochemical and Food Biotechnology, Bloemfontein, South Africa
[4]Lycoming College, Biology Department, Williamsport, United States
[5]National Collection of Type Cultures, London, United Kingdom
[6]Institut National de la Recherche Agronomique, Unité de Virologie et Immunologie Moléculaires, Jouy-en-Josas, France

Background: The genus *Chryseobacterium* is known to be polyphyletic, and there are few consistent phenotypic differences in this group of organisms.

Objectives: We sequenced twenty-eight strains from our collection and compared them to all publically available *Chryseobacterium* type strain genomes. While our initial goal was to determine the species classification of these strains, the study expanded to include classification of each monophyletic grouping as its own genus.

Methods: A multi-modal distribution was found among Amino-Acid Identity (AAI) values calculated on proteins that met the criteria of having 40% in amino acid identity and 50% in coverage length, when strains were compared in a pairwise fashion. These naturally occurring non-continuities were used to guide a standardization of *Chryseobacterium* classification.

Results: We propose transfer of ten species from the genus *Chryseobacterium* to the genus *Epilithonimonas,* eleven species to the genus *Kaistella,* and two species to the genus *Halpernia* gen. nov. Furthermore, evidence is presented that contradicts our earlier proposal to transfer *Planobacterium taklimakanense* to the genus *Chryseobacterium*.

We put forward a hypothesis that the origin of both genus and species distinctions result from a loss of biodiversity caused by the Permian and Cretaceous–Paleogene extinctions, respectively, followed by population expansion and biodiversity recovery once the environmental conditions leading to the extinction event ended.

Regardless of cause, use of AAI comparisons between strains to define those ≥76% as same genus or ≤74% as different provides a mechanism for future taxonomists to determine if a novel species belongs to one of these genera.

PT406 Genomic encyclopedia of bacterial and archaeal type strains, Phase VI: The functional genomics and pangenomes of type strains

William Whitman[1], Hans-Peter Klenk[2], David Arahal[3], Rosa Aznar[3], George Garrity[4], Markus Goeker[5], Michael Pester[5], Phil Hugenholtz[6]

[1]University of Georgia, Department of Microbiology, Athens, United States
[2]Newcastle University, School of Natural and Environmental Sciences, Newcastle upon Tyne, United Kingdom
[3]Universidad de Valencia, Colección Española de Cultivos Tipo (CECT), Valencia, Spain
[4]Michigan State University, Department of Microbiology and Molecular Genetics, East Lansing, United States
[5]Leibniz Institute DSMZ, German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany
[6]The University of Queensland, Australian Centre for Ecogenomics Research, Brisbane, Australia

Background: The Genomic Encyclopedia of *Bacteria* and *Archaea* (GEBA) project was launched by the JGI in 2007 as a pilot project to sequence about 250 bacterial and archaeal genomes of elevated phylogenetic diversity. During the following decade, GEBA projects have sequenced the genomes of more than 3,145 type strains.

Objectives: In GEBA VI this project is extended to include the functional genomics of the type and reference strains of understudied groups. The goal of these studies is to improve our understanding of gene function for a wide variety of prokaryotes utilizing the tools of genomics, pangenomics, transcriptomics and exometabolomics.

Methods: Two types of experimental designs are proposed. One, comparative studies of groups of related type strains, including surveys of genome sequences, transcriptomes and exometabolomes. Two, more in depth studies of a single species, including characterizations of the pangenome or transcriptomes and exometabolomes under different growth conditions. The resources allocated to this effort include Illumina sequencing of the genomes of 10,000 prokaryotes. These genomes will comprise 5000 type strains. The remainder will be 5-10 reference strains for determination of the pangenomes for 500-1000 species. In addition, the transcriptome and exometabolites in culture broths can be determined for up to 1000 type strains.

Results: Like the previous GEBA projects, investigators from any country are invited to submit samples. See https://gold.jgi.doe.gov/gebaVI for more details on the program, sample preparation, and instructions on how to participate in the project.

PT407 New polyene antibiotic roseofungin, active against the causative agents of candidal vulvovaginitis

Amankeldi Sadanov[1], Assya Balgimbaeva[1], Liliya Ibragimova[1], Aisha Sultanova[1], Mookhit Esirkepuly[1], Ramilya Galimbaeva[1], Gauhar Tugelbay[1], Erik Shorabaev[1], Zere Turlybaeva[1], Ludmila Trenozhnikova[1]

[1]LLP Scientific Production Center for Microbiology and Virology, Almaty, Kazakhstan

Background: Treatment and prevention of candidal vulvovaginitis is currently an urgent problem in gynecology. The polyene antibiotic roseofungin is a new drug substance. The purpose of this study was to examine the activity of the antifungal polyene antibiotic roseofungin against the clinical pathogens of vaginal candidiasis and assess the possibility of its use for the development of a new dosage form.

Objectives: The object of the study was a polyene antibiotic roseofungin.

Methods: Antifungal activity of the antibiotic roseofungin was studied by the agar diffusion method against 15 clinical strains of the genus *Candida*: 9 strains of *Candida albicans*, 2 strains of *Candida krusei*, 2 strains of *Candida tropicalis*, 1 strain of *Candida glabrata*, 1 strain of *Candida parapsilosis*.

Results: The biological activity of the antibiotic rozeofungin was examined against 15 clinical strains of the genus *Candida*. The minimum inhibitory concentration of the studied strains varied in the range of 1.66-2.5 microgram per milliliter (μg/mL). The highest activity of the antibiotic roseofungin was observed against clinical *Candida albicans* strains, minimum inhibitory concentration values were within the 1.66-2.0 μg/mL range. Activity against *Candida non-albicans* strains was lower: minimum inhibitory concentration of 2.0 μg/mL for *Candida tropicalis*, and 2.5 μg/mL for *Candida krusei, Candida glabrat,* and *Candida parapsilosis*. High activity of the antibiotic roseofungin against clinical pathogens of candidal vulvovaginitis has shown promise for developing a new drug on its basis.

PT408 Introduction of the DR1558 gene into the engineered Corynebacterium glutamicum strain for improved cadaverine production.

Soongbin Kang[1], Jong-il Choi[1]

[1]Chonnam National University, Biotechnology and Bioengineering, Interdisciplinary program for Bioenery & Biomaterials., Gwangju, Korea, Rep. of South

Background: Previous studies have shown that the *DR1558* gene from *Deinococcus radioduransin*, it can regulate multistress if introduced into a strain. The engineered *Corynebacterium glutamicum ldc* strain is a cadaverine producing strain. In this study, the *DR1558* was introduced into the engineered strain to confirm the improved production of cadaverine at low pH.

Objectives: To express *DR1558* from *Deinococcus radioduransin* in an engineered *Corynebacterium glutamicum* KCTC 1857 *ldcC* strain, an cadaverine producing strain*, DR1558*, was integrated in to the strain by gene cloning and controlling its expression with a strong synthetic H30 promoter**.** It was confirmed that the newly constructed strain had more cadaverine production due to the *DR1558* gene**.**

Methods: In order to compare the amount of cadaverine produced under the low pH condition due to the *DR1558* gene, the strain was cultured at various pH conditions to confirm the amount of cadaverine production. A control strain was used in which strain *DR1558* was not introduced. The total cadaverine production was confirmed by HPLC.

Results: The strains in which *DR1558* was introduced than the wild type strains showed higher production of cadaverine at lower pH and higher cell growth. After 48 hours of incubation, the residual lysine was found to be smaller than wild type strain. As a result, we have shown that *DR1558* gene can be expressed as a stress-regulated gene in the engineered-strain to produce more targeted products.

PT409 Alterations of the Gut Microbiota for Assessing the Hazard of Nanomaterials

Matthew Meier[1], Kathy Nguyen[2], Jennifer Crosthwait[2], Alice Kawata[2], Marc Rigden[2], Karen Leingartner[2], Alex Wong[3], Alison Holloway[4], Phil Shwed[2], Lee Beaudette[1], Martha Navarro[5], Michael Wade[2], Azam Tayabali[2]

[1]Environment and Climate Change Canada, Biological Assessment and Standardization Section
[2]Health Canada, Environmental Health Sciences and Research Bureau
[3]Carleton Unversity, Department of Biology
[4]McMaster University, Department of Obstetrics and Gynecology, Hamilton, Canada
[5]Health Canada, Bureau of Chemical Safety

Background: The gut microbiota is linked to human health. Its disruption through exposure to numerous chemicals is associated with metabolic, immunological, and neurological diseases. The complex dynamics of gut microbiota and its processes implicate it as a potential target of toxicity. Yet, there is insufficient knowledge about how changes in gut micobiota composition can inform hazard characterization and risk assessment.

Objectives: This study investigated if silver nanoparticles (AgNPs) disrupt mouse gut microbiota, and if this dysbiosis could be used to evaluate hazard associated with nanomaterial exposures.

Methods: Mice were weighed and exposed daily for 5 weeks by gavage to either saline, AgNPs or antibiotics, or co-exposed to AgNPs with antibiotics. Animals were assessed for glucose tolerance, organ function, and immunotoxicity. Tissue distribution of silver was also determined. Structure and function of the caecum and feces microbiota were evaluated by 16S rRNA and shotgun sequencing.

Results: Mice exposed to antibiotic alone demonstrated reduced response to glucose challenge, but marginal alterations in the composition of gut microbiota. There were also minimal effects from exposure to AgNPs alone. In contrast, AgNPs and antibiotic co-exposure caused reduced body weight, lymphopenia, and splenic inflammation. Co-exposure also resulted in microbiota dysbiosis and a striking increase in the Firmicutes to Bacteroidetes ratio compared to animals treated with either saline, antibiotic alone, or AgNP alone. This study demonstrated that toxicological effects and microbiota dysbiosis due to AgNPs were compounded by antibiotic exposure. Although preliminary, these results suggest that gut microbiota perturbations may increase vulnerability to hazardous nanomaterials.

PT410 Waste not, Want not: Enhancing the Ability of Yeast to Utilise its Own Leftovers from the Brewing Industry to Fuel the Transportation Industry with Ethanol.

Ainsley Beaton[1], Nick Tucker[1], Franck Escalettes[2], Leonardo Magneschi[2]

[1]*Strathclyde Institute of Pharmacy and Biomedical Sciences, Glasgow, United Kingdom*
[2]*Ingenza Ltd, Edinburgh, United Kingdom*

Background: The brewing industry produces a large amount of waste, 85% of this is composed of spent brewers' grain. One use for this bi-product is in the bioethanol industry where the yeast, *S. cerevisiae* uses the spent grain as a feedstock. Due to the nature of the feedstock, there is a lack of utilisable carbon for *S. cerevisiae.*

Objectives: To obtain optimum utilisation of this feedstock in conjunction with high process efficiency by enhancing carbon metabolism of the production strain. As well as expanded nutrient utilisation there is also a requirement to maintain the high ethanol production and tolerance that industrial strains have acquired in a preferred growth medium.

Methods: We are using high-throughput phenotypic arrays to rapidly identify strains best able to grow in a wide range of conditions, including various carbon and nitrogen sources and multiple stress inducing conditions. In collaboration with an industrial partner, both targeted and random chromosomal integration of transgenes have been made to multiple candidate production strains. In addition, whole genome sequencing is being utilised to interrogate the genetic basis for phenotypic differences between production strains.

Results: Phenotypic array's have shown small but measurable differences between production strains in industrially relevant growth conditions. Changes can be observed through iterations of gene insertions which improve recycled feedstock utilisation and process efficiency. Modifications are being validated by whole genome sequencing which has shown that some important null phenotypes are at the transcription level, this information is now in use to drive future rounds of genetic manipulation.

PT411 Bacteria:the tiniest story ever told

Paola Scavone[1], Vanesa Amarelle[2], Daniela Arredondo[1], Gastón Azziz[2], María José González[1], María Morel[2], Nicolás Peruzzo[3], Alejandro Rodríguez Juele[3], Ana Umpiérrez[1]

[1]Instituto de Investigaciones BiolOgicas Clemente Estable, Departamento de Microbiología, MONTEVIDEO,
[2]Instituto de Investigaciones BiolOgicas Clemente Estable, Departamento de Bioquímica y genómica microbiana, MONTEVIDEO,
[3]Bandas Educativas, Uruguay

Background: Throughout history and in the popular culture, bacteria have been mainly associated with human and animal disease. However, bacteria are essential for life on Earth. They are present in our daily lives and provide us with a seemingly infinite stock of resources. Convinced of the importance that education in science has on children, we embarked in a project whose main objective was to promote the popularisation of microbiology.

Objectives: The goal was the creation of a comic book that explains the wonderful and amazing world of bacteria in a fun and entertaining way, thereby bringing microbiology close to the public, particularly to children.

Methods: Coco and Frank are two bacteria that live in “Bacteritown”, located at the coffee table of the living room of a family house. On a visit to the bacterial museum, Coco and Frank meet the museum janitor, which informs them that the “Great Disinfection”, a cataclysm that eliminated 99.9% of Bacteritown inhabitants, will happen again soon. Coco and Frank undertake a journey in search for one of the survivors, Ancyanobacterium, and his wisdom to avoid the cataclysm. This expedition takes them through the human gut, water, and soil, where they will meet different bacteria and learn about their distinctive features. Throughout their journey, Coco and Frank will live moments of tension but also moments of great fun.

Results: The printed version was distributed to public schools in Uruguay. The comic (soon in English) is freely available at http://www.bandaseducativas.com. Two associated gamesare also available for download.

PT412 Comparative analysis of the change in the composition of Diabetic Foot Ulcer microbiomes in response to different therapies

Thomas Walker[1], Janice Spencer[1]

[1]*Glasgow Caledonian University, Health and Life Sciences, Glasgow, United Kingdom*

Background: Diabetic foot ulcers (DFUs) are slow healing wounds which arise from co-morbidities associated with diabetes. Often these ulcers become infected leading to gangrene, osteomyelitis and sepsis. Therapies currently include debridement and a topical irrigant which have limited success. Understanding the effects that these treatments have on DFU microbiomes and on wound healing is poorly understood.

Objectives: To evaluate the effects of two wound irrigants on DFU microbial composition and DFU healing

Methods: 16S rRNA sequencing was used to identify the microbial composition of 27 DFU samples from 7 patients after treatment with one of two irrigant solutions (Prontosan and Electrolysed water - E.W.) . Fluorescence and conventional biofilm assays were performed to assess the effectiveness of the two irrigants in vitro.

Results: Across the 27 samples commonly observed genera present included *Staphylococci (2*6/27), *Propionibacterium* (26/27) and *Finegoldia* (24/27). Increasing microbial diversity within the DFUs was correlated with an increased percentage abundance of anaerobic and Gram negative genera and inversely correlated with facultatively anaerobic and Gram positive genera. No significant reduction in diversity or species richness of the DFU microbiomes was observed after treatment with either irrigant.

Both Prontosan and E.W. had similar effects upon *S.aureus* biofilms reducing viability by 82.013% and 86.89% respectively however E.W. efficacy was strain specific. In addition, E.W. was ineffective at preventing biofilm formation in 6/8 *S.aureus* strains. Better understanding of the DFU microbiome and investigations into novel therapies is paramount to aid our ability to improve the quality of life for diabetic patients.

PW001 Bioaugmentation and phytoremediation: an integrated approach for soil remediation

Maria Simona Zangrillo[1], Eren Taskin[1], Giulia Spini[1], Pietro Iavazzo[2], Simone Anelli[2], Lucrezia Lamastra[1], Nicoleta Suciu[1], andrea ferrarini[3], Stefano Amaducci[3], Marco Trevisan[1], Edoardo Puglisi[1]

[1]Università Cattolica del Sacro Cuore, Dipartimento di Scienze e Tecnologie Alimentari per una filiera agro-alimentare Sostenibile - DiSTAS, Piacenza, Italy
[2]Ente Regionale per i Servizi all'Agricoltura e alle Foreste - Ersaf, Brescia, Italy
[3]Università Cattolica del Sacro Cuore, Dipartimento di Scienze delle Produzioni Vegetali Sostenibili, Piacenza, Italy

Background: Worldwide soils are widely contaminated by pollutants and bioremediation can provide a viable option for the soil restoration. Bioaugmentation combined with phytoremediation was applied in this work for the removal of pollutants.

Objectives: To use indigenous microorganisms in combination with plants to remediate contaminated site in Mantova Municipality (Italy) where the measured levels of Polycyclic Aromatic Hydrocarbons (PAH) and mercury were slightly higher than the limits allowed in Italy for the residential areas.

Methods: Enrichment of bacterial and fungal autochthonous species were carried out using soil samples taken from the contaminated site, using the PAHs *pyrene*, *benzo(b)fluorantene* and *benzo(ghi)perylene* as sole carbon sources. The fungi and bacteria that were able to use the above-mentioned PAHs as carbon source were then isolated, identified and selected based on the best degradation efficiencies. *Stenotrophomonas maltophilia*, *Fusarium solani* and *Hypochnicium bombycinum* resulted the best degraders. Biosurfactant production capacity was also evaluated. Microbial consortium was then tested for its performance associated with following plant species in greenhouse conditions: *Arundo donax*, *Festuca arundinacea*, *Populus alba* + *Festuca arundinacea* and *Salix alba*. The same consortium was used for the field experiments with following plants: *Arundo donax*, *Festuca arundinacea*, *Populus alba* + *Festuca arundinacea*, *Populus alba* + *Trifolium pratense*.

Results: It was observed that pyrene, *benzo(b)fluorantene* e *benzo(ghi)perylene* were best degraded by the *Stenotrophomonas maltophilia* bacterium and *Fusarium solani* and *Hypochnicium bombycinum* fungi. The degradation efficiencies of the microbial consortiums both in the greenhouse and field conditions is now being determined and will be presented.

PW002 Microbial community structure of full scale anaerobic digester systems in Dubai, UAE

Munawwar Ali Khan[1]

[1]*College of Natural and Health Sciences, Zayed University, Life and Environmental Sciences, Dubai, United Arab Emirates*

Background: Anaerobic digestion is a commonly used method for wastewater sludge stabilization for various reuse applications to reduce the impact of the organic pollutants on the environment. Anaerobic digestion of municipal wastewater sludge depends on an exceptionally diverse microbial consortium. Acquiring knowledge of microbial population functions, dynamics, and behavior is necessary for the process control and optimization of anaerobic sludge digester systems.

Objectives: To study microbial communities of three anaerobic digester systems of a full-scale municipal wastewater treatment Plant in Dubai, UAE.

Methods: The microbial community DNA was extracted using PowerSoil DNA isolation kit from MoBio laboratories according to manufacturer instructions. Next generation Sequencing (NGS), quantitative real-time Polymerase Chain Reaction (qPCR) and fluorescence *in situ* hybridization techniques were used to study relative abundance and diversity of microbial communities.

Results: Bacterial community was dominating over the archaeal community in all three anaerobic digesters. qPCR analysis showed that the bacterial community was found to be in the range of $1.04x10^9$ to $7.94x10^9$ copy number per microliter of sample whereas the archaeal population was found to be in the range of $1.5x10^6$ to $12.1x10^6$ copy number per microliter. qPCR and NGS analysis showed that the order *Methanomicrobiales* was dominating in all the digesters followed by the order *Methanosarcinales* and the *Methanobacteriales* respectively. In conclusion, qPCR and NGS analysis provided overall mapping of the bacterial and archaeal community structure of anaerobic digester systems.

PW003 Data integration to correlate microbial activity to molecules degradation during anaerobic digestion

Laëtitia Cardona[1], Kim Anh Lê Cao[2], Chrystelle Bureau[1], Céline Madigou[1], Lénaïck Rouillac[1], Laurent Mazéas[1], Olivier Chapleur[1]

[1]irstea, hydrosystems and bioprocesses, Antony, France
[2]Melbourne University, Melbourne Integrative genomics research unit

Background: Anaerobic digestion (AD) is a promising process providing sustainable energy drove by a complex microbial community. However little is known about the microbial activity, their interactions or structuration which makes of AD a bioprocess still unstable. Data integration of different level of information regarding the microbial activity is a promising way to decipher the biological mechanisms behind AD.

Objectives: The objective of this work is to propose a strategy to identify correlations between active microorganisms and the molecules degradation occuring in the bioprocess.

Methods: 9 different feeding compositions were used to feed bioreactors in triplicate (fish, grass or sewage sludge in mono-digestion and 3 linear mixes of sludge:grass and sludge:fish). 16SrRNA sequencing and metabolomic analysis were carried out at the beginning and during the active production step. Integrative biostatistical tool (canonical Partial-Least-Square, mixOmics R package) was used to correlate the active microorganisms to the molecules degradation.

Results: Multivariate analyses were applied to highligh the key microorganisms and molecules involved in the different bioreactors. We have shown that feeding composition influenced their dynamic evolutions. Morevoer specific feeding condition induced the activity of some microorganisms as Methanocorpusculum active in only two mixes of grass:sludge.

By applying data integration between microbial activity and molecules degradation rate we have correlated the microorganisms to the degradation of the molecules. For example, cadaverine, identified during fish mono-digestion, was correlated to Methanosarcina and several Clostridiales suggesting their role on cadaverine degradation. This study provides new perspectives on anaerobic microbiota analyses using data integration.

PW004 comparative genomics of endosymbiotic bradyrhizobia of arachis hypogea and endophytic bradyrhizobia of endophytic bradyrhizobia of coryza sativa

Sohini Guha[1], Beatrice Jorrin[2], philip poole[2], monolina sarkar[1], maitrayee dasgupta[1]

[1]*University of Calcutta Department of biochemistry, Kolkata, India*
[2]*Department of Plant Sciences, University of Oxford, plant sciences, Oxford, United Kingdom*

Background: The family of Bradyrhizobiaceae is composed of members with diverse habitats. As reported earlier, two distinct categories of *Bradyrhizobium-WBAH(West Bengal Arachis hypogaea)* and WBOS(*West Bengal Oryza sativa*) colonize *Arachis hypogaea* and *Oryza* in an *Arachis-Oryza* intercropped field, despite having comparable modes of entry via epidermal cracks.

Objectives: In this study draft genomes of 6 *WBAH* and 6 *WBOS* strains was produced using Illumina Miseq ,in order to study their genomes.

Methods: Comparative genome analyses was done to identify gene families and processes associated with the WBAH-WBOS, to understand the molecular underpinning of their selective host associations.

Results: The WBAH and the WBOS strains differed considerably in their genomic features. The average nucleotide identities placed the WBAH strains within *Bradyrhizobium yuangmingense* group while the WBOS strains had no known relatives. The WBOS strains suffered extreme genome reduction. The WBAH strains had the canonical *nod-nif-fix* loci while the WBOS lacked them. The pan-core data analyses predicted a high intraspecific diversity of gene repertoires, affecting major ecological principles like Root Nodule Symbiosis (RNS) that may explain the observed association of the strains. Our findings contribute to an increasingly detailed portrait of genomic features defining the biology of the genus *Bradyrhizobium*.

PW005 Disparity in Hand Hygiene Perception and Practice Attributes to Drug Resistant Pathogens Among School Children of Karachi, Pakistan

Javeria Samad[1], Anabia Alam[2], Sidra Sikandar[1], Humaira Qureshi[1]

[1]*Habib University, ISciM, School of Science and Engineering, Karachi, Pakistan*
[2]*Habib University, School of Science and Engineering, karachi, Pakistan*

Background: School children often catch communicable diseases due to poor hand hygiene. Their close interaction, lack of awareness and substandard hand washing facilities contribute to contaminated hands.
Objectives: This cross sectional study was performed to correlate the hand washing perception among school children and the actual bacterial load present on their hands.

Methods: Students (n=61, grades 6^{th} – 8^{th}) from five schools of lower to lower-middle class were randomly selected from Karachi. A question based survey was conducted to evaluate their understanding of basic hand hygiene. To check the bacterial load, hands of children were swabbed by sterile cotton swabs. The samples were inoculated on differential and selective media. Plates were incubated for 24 hours and results were confirmed by growth, morphology and biochemical tests. Antibiotic susceptibility was measured using Kirby Bauer test for all isolates.

Results: Survey revealed that students' perception regarding hand washing was above average; 72% agreed to washing hands before consuming food and after using restroom. In contrast, lab testing revealed that 61% children were not using soap after restroom usage. Results showed that children's hands were covered by *Bacillus spp*. (50%), *Listeria* (74%), *Staph. aureus* and *Micrococcus* (77%), *Pseudomonas* (54%) and *Staph. epidermidis* 86%. Only one sample had *Proteus*. Antibiotic susceptibility testing exhibited resistance to Ceftriaxone (60%), Augmentin (60%), Amoxicillin (70%) and Ciprofloxacin (20%).

This study showed the presence of pathogenic bacteria on children's hands that could lead to outbreaks of drug resistant organisms. It reveals an inconsistency in perception versus practice of hand hygiene.

PW006 Co-occurrence of heavy metals and extended-spectrum beta-lactamase resistances genes in Enterobacteriaceae strains isolated along the whole course of a river in Romania

Luminita Gabriela Marutescu[1], Marcela Popa[1], Irina Gheorghe[1], Ilda Czobor[1], Andreea Cretu[1], Carmen Curutiu[1], Ioan Pacesila[2], Mariana Carmen Chifiriuc[1]

[1]*Faculty of Biology, University of Bucharest, Bucharest, Romania*
[2]*Institute of Biology, Bucharest, Romania*

Background: Antibiotic resistance (AR) has become a global concern, leading to serious limitations of bacterial infections treatment options. AR bacteria and resistance genes (ARGs) have been detected in natural aquatic environments, but their fate and persistence are still largely unknown, particularly in different geographical areas. Heavy metals represent a major source of environmental contaminants that may select for AR.

Objectives: to investigate the association of heavy metal genes (HVGs) in beta-lactam resistant Enterobacteriaceae isolated along the whole course of the Argeș River, in Romania.

Methods: Surface water samples were processed by membrane filtration, using ECC agar for E. coli and ChromID ESBL agar for ESBL-Enterobacteriaceae. The recovered resistant colonies were identified by MALDI-TOF MS and their resistance profile by VITEK 2. ARGs and HVGs were detected by PCR.

Results: The Enterobacteriaceae isolates recovered from ChromID ESBL agar were resistant to: ampicillin and ceftazidime (100%), cefotaxime and cefepime (96.9%), ciprofloxacin (75.75%), sulfamethoxazole-trimethoprim (51.51%) gentamicin (24.44%) and meropenem (12.12%). The blaTEM, blaCTX-M, and blaSHV (beta-lactam), aadA1aadB, aacC1, aphA1,aphA6 (gentamicin), sul1, sul2 (sulfonamide), dhfrI (trimethoprim), qnrA, qnrB and qnrS (quinolone) ARGs were detected. Of the three targeted HVRs, pcoA for Cu2+ was detected in 15.15% of the ESBL-positive isolates, all possessing the blaCTX-M, blaSHV, and blaTEM genes. **Conclusion.** Our results revealed the co-occurrence of clinically important ARGs and HVGs in acquatic bacterial strains, demonstrating the urgent need for studies trying to unravel the linkage among heavy metal concentration, bacterial community composition, ARG abundance and their genetic mobility in the water reservoir.

PW007 Aerobic oral microbiota in Romanian institutionalized frail elderly with removable dentures

madalina bicheru[1], Luminita Marutescu[2], Cristina Preoteasa [1], Elena Preoteasa[1], Madalina Maria Muntean[1], Carmen Mariana Chifiriuc[2], Marcela Popa[2]

[1]*Carol Davila University of Medicine and Pharmacy, Bucharest, Romania*
[2]*Faculty of Biology, University of Bucharest, Bucharest, Romania*

Background: While investigation of the microbiome on natural oral surfaces has generated a wealth of information, few studies have examined the microbial communities colonizing dentures and their relationship to oral health in frail elderly.

Objectives: to describe the diversity of the microbial communities colonizing dentures and palatal surface of the elderly, exhibiting xerostomia.

Methods: The study was conducted on 30 patients with removable dentures with xerostomia, recruited from a nursing home. Saliva samples were collected from the internal surface of the denture and hard palate mucosa and inoculated on different media (blood agar, Chapman, bile esculine agar, Chromagar ECC, Sabouraud, Chromid OXA 48, Chromaid ESBL). MALDI-TOF mass spectrometry was used for the identification of recovered bacterial and fungal strains. Microbial virulence features were evaluated by plating the strains on agar supplemented with different enzymatic substrate for the assessment of haemolysis, lipase, lecithinase, proteases, esculine hydrolysis and by evaluating the adherence pattern to human cell standard line.

Results: The aerobic cultivable microbiota was very diverse, the most prevalent bacterial genera being *Staphylococcus* spp., *Streptococcus* spp., *Lactobacillus* spp., *Klebsiella* spp., *Actinobacter* spp., *Enterobacter* spp., *Pseudomonas* spp., while *Candida* spp. strains were isolated from 90% of the investigated patients. Medication and systemic disease have significantly influenced the oral microbiota diversity and abundance in the study group. The isolated microorganisms exhibited different profiles of soluble and cell-associated virulence factors. Knowledge regarding specific patterns of the oral microbiota may provide additional diagnostic tools in denture wearing patients with xerostomia.

PW008 Antimicrobial and plant-growth promoting ability of Pantoea agglomerans isolated from endosphere of wheat (Triticum aestivum)

Katsiaryna Salauyova[1], Zinaida Aleschenkova[2]

[1]*Institute of microbiology, Minsk, Belarus*
[1]*Institute of microbiology, Minsk, Belarus*

Background: Genus *Pantoea* belonging to the family *Enterobacteriacea* was first described by Gavini et al. in 1989. Bacteria constituting the genus are Gram-negative motile rods generating as a rule the yellow pigment. Some representatives of genus *Pantoea* isolated from internal plant tissues display a variety of beneficial properties (nitrogen-fixing ability, mobilization of hardly soluble compounds, synthesis of antibiotics, etc.) and hence are regarded as promising biotechnological agents.

Objectives: Aim of our study was investigation of endophytic bacterial community colonizing wheat crop cultivated in Belarus and screening of microbial strain with characteristics valuable for plant host.

Methods: Endophytic bacteria were isolated from roots, stems, leaves and seeds of winter wheat (*Triticum aestivum*), variety Mogilevskaya tilled at local fields. Endophytes were recovered by surface sterilization method with preliminary optimization of the procedure. The scheme of sterilizing surface of plant specimens comprised treatment with 3% hydrogen peroxide solution.

Results: 102 isolates of endophytic bacteria were recovered from vegetative specimens of wheat. The most active culture 6SK possessing the maximum phosphate-solubilizing, growth-promoting, antimicrobial, colonizing capacity was screened and further identified as *Pantoea agglomerans* based on morphological, physiological-biochemical and molecular-genetical characterization. The selected strain showed antimicrobial action against phytopathogenic bacteria of genera *Pseudomonas* and *Xanthomonas*. Treatment of wheat seeds with strain *Pantoea agglomerans* 6SK stimulated seed germination, increased stem height and root length in comparison with non-inoculated plants under model conditions. Summing up, the isolated strain of endophytic bacteria *Pantoea agglomerans* 6SK is extremely attractive for application as biocontrol and plant growth-promoting (BPGP) agent.

PW009 Diversity of microbial community in the urban wetland Santa María del Lago, Bogotá, Colombia

Maribeb Castro[1], Mauricio Pacheco[2], Mariana Restrepo[2]

[1]*Universidad Militar Nueva Granada, Facultad de Ciencias Bàsicas y Aplicadas, Cajica, Colombia*
[2]*Universidad Militar Nueva Granada, Facultad de Ciencias Básicas y Aplicadas, Cajicá, Colombia*

Background: The complex of urban wetlands in the Bogotá city is of at least 667 hectares and it has been included in the Ramsar list for the conservation of its fauna and flora. One of the most important and conserved is the Santa María del Lago wetland, which has been characterized by its temporal and spatial variability in nutrient levels and mesotrophic-eutrophic conditions along the year. However, there is not studies about of microbial communities composition in this area despite of its importance for nutrients and greenhouse gases cycling.

Objectives: The goal of this research was explore the spatial and seasonal composition of microorganisms in sediments of this wetland.

Methods: The community was studied using 16S rRNA Illumina Miseq sequencing and phylogenetic analysis along with correlation and correspondence analysis to evaluate the effect of spatial and seasonal environmental factors on the composition of microorganisms.

Results: The Proteobacteria, Acidobacteria and Aminicenantes were the main phylum founded for bacteria and Methanomicrobia and Thermoprotei for Archaea. The phylogenetic analysis suggested a high diversity of OTUs some which were similar to the reported for other sediments and soils. The highest abundance and diversity of phyla was observed during the dry season and its composition was strongly correlated with acidic pH (6.6), high Cd, Cu, Zn, Fe and Pb (correspondence analysis), and low levels of organic carbon, phosphorus and ammonium (Pearson analysis).

PW010 Appraisal of wastewater final effluents as reservoirs of cholera and non-cholera Vibrio species: A case study of the two wastewater treatment plants in Amatole and O.R Tambo District Municipality, South Africa

Nolonwabo Nontongana[1], Anthony Okoh[1]

[1]*Alice, Biochemistry and Microbiology, Alice, South Africa*

Background: In the last decade, Vibrio disease outbreaks have created a painful awareness of economic, societal, and public health costs associated with the impact of contaminated water in the aquatic milieu.

Objectives: This study was designed to assess the prevalence of Vibrio pathogens in the final effluents of two wastewater treatment plants (WWTPs) in the Eastern Cape Province (MT and BT).

Methods: All samples were collected (December 2016 to November 2017) aseptically using sterile 1L glass bottles and transported on ice to the laboratory for analyses within 6hrs of collection. The membrane filtration method was used for enumeration of presumptive Vibrio densities on thiosulfate citrate bile salt agar plates. Polymerase chain reaction was then used to confirm the identities of the presumptive Vibrio species, using species-specific primers. The confirmed isolates were further subjected to molecular characterization to confirm their respective pathotypes.

Results: Presumptive Vibrio densities varied from 2.91 to 3.91 $\log_{10}$ CFU/100ml and 2.67 to 3.18 $\log_{10}$ CFU/100ml, for BT and MT WWTPs, respectively. Out of 720 presumptive isolates, 86% were positive for the Vibrio genus. The recovery of Vibrio in these effluents throughout the sampling period even in adequately disinfected effluents is unacceptable considering the fact that Vibrio is a pathogenic bacterium. The findings of this study underline the need for constant monitoring of the microbiological qualities of discharged effluents and also suggestive for a review of the disinfection methods used as this might pose adverse health risk to the communities which still rely heavily on these surface waters.

PW011 Increase on the temperature in the Colombian Andes: Monitoring of the morphological characteristics of bacteria present in the paramo from the National Park "Los Nevados"

Nilza Velasco[1], Patricia Velez[1;2], Ariane Coral[1], Bibiana Duarte[1;2], Pedro Moreno[3]

[1]Universidad del Cauca, FACNED, Departamento de Biología, Popayán, Colombia
[2]Cauca Universidad del Cauca, Centro de Excelencia en Genómica y Bioinformática, GeBiX, Colombia
[3]Universidad del Valle, Facultad de Ingenieria, EISC, Cali, Colombia

Background: Colombia is crossed by 3 large mountain ranges of the Andes called Western, Central and Eastern Cordillera where many ecosystems are located, including the paramo. The present study was conducted in a paramo located at an altitude of more than 3000 masl, which has a temperature that fluctuates between -2 and 4 °C, specifically the National Natural Park "Los Nevados" (PNNLN), this park is located between 2600 - 5300 masl in the Colombian Andes, it has high-andean forest, paramo and nival ecosystems, a temperature that fluctuates between -3 to 14 °C, where numerous studies have been carried out in the so-called "GeBiX Project" (Platform for the Characterization and Analysis of Organisms of Extreme Environments).

Objectives: We want to identify and analyze the bacteria morphology that are part of the microbiological communities on soils of Colombian paramos ecosystem.

Methods: We used different techniques of microbiological isolation and morphological analysis of some present bacteria

Results: The structures are characteristic of the extreme climatic conditions of these paramos and the morphological changes observed in the different samplings could be the result of climate change. These observed morphological characteristics had not previously been analyzed in these paramos of the Colombian Andes; therefore, this work constitutes a new reference for the microbiological study of the soils of these ecosystems and may serve as comparative parameters for monitoring this and other paramos in Colombia (and other regions of the world), especially when temperatures are currently increasing, with a possible impact on these ecosystems.

PW012 Potential of newly isolated thermotolerant yeast Klyuveromyces marxianus JKSGH-6 in enhancing cellulosic ethanol production

Hemansi Prasad[1], Garima Yadav[1], Jitendra Saini[1]

[1]*Central University of Haryana, Microbiology, Mahendergarh, India*

Background: Lignocellulosic biomass (LCB) is a renewable and environmentally friendly feedstock for sustainable production of biofuels and other valuable chemicals. However the major challenges are biomass recalcitrance and its efficient and economic bioconversion. Simultaneous saccharification and fermentation (SSF) is often employed for bioethanol production for enhancing the efficiency by application of thermotolerant ethanologenic yeast.

Objectives: Main aim of the study was the application of thermotolerant yeast for efficient bioethanol production of dilute alkali pretreated cotton stalk (CS).

Methods: Dilute alkali pretreatment (DALP) of milled 10% dry wt., CS (40-60 mesh size), was carried out at 121 °C for 30 min using NaOH (3%; w/v). Thermotolerant yeasts were screened for ethanol production at 42°C and the best isolate was identified by amplification and sequencing of ITS-5.8s rRNA gene and used in SSF of pretreated CS.

Results: Under SSF of DALP-CS at 42°C strain produced ethanol titer of 39.4 g/L. The enzymatic hydrolysis of DALP-CS, using commercial enzyme, released 204 mg/g of sugar. Cellulose content of DALP-CS increased to 73% (w/w) than that in native (50%; w/w) CS due to lignin removal which was confirmed by the changes in absorption spectrum during Fourier-transform infrared spectroscopy (FTIR) analysis. Out of 15 thermotolerant (growing at 42°C) isolates screened, the best ethanol fermenting isolate was identified as *Klyuveromyces marxianus* JKSGH-6. The newly identified strain *K. marxianus* JKSG-6 has potential for better ethanol production from CS and other LCB.

PW013 Bioaugmentation with bacteria selected from the microbiome enhances the growth of Arthrocnemum macrostachyum in polluted marshes

SALVADORA NAVARRO-TORRE[1], Jose M. Barcia-Piedras[2], Miguel Angel Caviedes[1], Eloisa Pajuelo[1], Susana Redondo-Gómez[3], Ignacio D. Rodriguez-Llorente[1], Enrique Mateos-Naranjos[3]

[1]*Universidad de Sevilla, Microbiologia y Parasitologia, Sevilla, Spain*
[2]*IFAPA, Centro las Torres-Tomejil, Sevilla, Spain*
[3]*Universidad de Sevilla, Biología Vegetal y Ecología, Sevilla, Spain*

Background: *Arthrocnemum macrostach*yum is a halophyte naturally growing in southwest coasts of Spain that can tolerate and accumulate heavy metals. In a previous work, 48 bacteria were isolated from *A. macrostachyum* growing in the Odiel River marshes, an ecosystem with high levels of contamination. Two bacterial *consortia* with the best-performing endophytic or rhizospheric strains were selected on basis on the heavy metals resistance and PGP properties.

Objectives: The aim of this study was to investigate the role of isolated bacteria of *A. macrostachyum* in the seeds germination, its metal uptake capacity and tolerance to metal excess.

Methods: A germination kinetics was observed in presence and absence of heavy metals using two bacterial *consortia* isolated from the rhizosphere (CR) and endosphere (CE) of *A. macrostachyum*. On the other hand, a glasshouse experiment was designed to investigate the role of bacterial consortia on its metal uptake capacity and tolerance in plants grown in metal polluted sediments. Plants were assigned to three treatments (with CR, CE and without inoculation).

Results: Bacterial inoculation accelerated germination of *A. macrostachyum* seeds in both the absence and presence of heavy metals. Bioaugmentation with both bacterial consortiums enhanced *A. macrostachyum* capacity to accumulate ions in its roots. Furthermore, bioaugmentation ameliorated the phytotoxicity levels. Bacteria had a beneficial effect on photochemical apparatus and on the modulation of its oxidative stress machinery. These results suggest that inoculation of *A. macrostachyum* with the selected bacteria could ameliorate plant establishment and growth in contaminated marshes and improve the metal remediation efficiency.

PW014 Deciphering Microbial diversity of Wheat Rhizosphere of Eastern Indo Gangetic Plain through metagenomics

Ruchi Srivastava[1;2], Anchal K. Srivastava[1], Promod W Ramteke[2], Vijai K Gupta[3], Alok K Srivastava[1], Anil K Saxena[1]

[1]ICAR-National Bureaue of Agriculturally Important Microorganism Kushmaur, Mau, India
[2]Sam Higginbottom University of Agriculture, Technology and Sciences, Allahabad, India
[3]Tallin University of Technology, Department of Chemistry and Biotechnology, ERA Chair of Green Chemistry, Estonia

Background: The microbial communities of the rhizospheric region of crop plants play a key role in plant growth promotion, productivity and health and nutrient cycling.

Objective: The present study is focused on the rhizospheric microbial diversity analysis of wheat, one of the major crop in India, through metagenomic approach.

Methods: The wheat rhizospheric soil from different regions of Eastern Indo Gangetic Plain,(EIGP) i.e. Mau (25.581285 N 83.592305 E), Ghazipur (25.913824 N 83.529715 E) and Ballia (25.778132 N 84.048146 E) were subjected to metagenomic analysis using Nanopore MinION and were compared with the published wheat rhizosphere metagenome (SRR924068) from Australia.

Results: The results uncovered that dominant phyla were Proteobacteria (64.78%), followed by firmicutes (12.46%), actinobacteria (4.87%), bacteroidetes (2.70%), acidobacteria (2.42%), chloroflexi (1.90%), cyanobacteria (1.03%), and gemmatimonadetes (0.93), whereas, in the Australian soil, the microbial community was recorded as, proteobacteria (33.33%), followed by Actinobacteria (26.70%), unclassified (derived from bacteria (16.55%), bacteroidetes (12.65%), Chloroflexi (6.33%), firmicutes (1.76%) and Acidobacteria (1.34%) Genus level comparison exhibited dominance of *Bacilli, Sphingomonas,* and *Pseudomonas* in EIGP samples, whereas, in Australian metagenome unclassified (derived from bacteria), *Streptomyces* and *flavobacterium* are dominant. Presence of *Gemmatimonadetes* indicates moisture stress in EIGP soils. The study will be helpful in developing microbe based technologies for abiotics stress and nutrient management in wheat. Similar to the SRR924068 metagenome of Australia, in present study also unclassified bacteria contribute about 16.55% of population that needs to be further analyzed for functions and interactions in the rhizosphere.

PW015 Activation of cryptic antimicrobial compounds by microbial co-cultures

Matthieu Nicault[1;2], Cyril Bontemps[1], Eric Gelhaye[2], Pierre Leblond[1]

[1]*umr 1128 dynamic, Meurthe et moselle, Vandœuvre-lès-Nancy, France*
[2]*umr 1136 iam, Meurthe et moselle, Vandœuvre-lès-Nancy, France*

Background: Natural antimicrobial molecules such as antibiotics and antifungals have profoundly changed the modern medicine and improved human health. However, with the increasing emergence of multiresistant pathogenic bacteria, there is nowadays a need to identify new molecules and antimicrobial activities.

Microorganisms constitute one of the principal source of bioactive natural products and, for instance, about half of the antibiotics used in the human medicine comes from the sole bacterial genus *Streptomyces*. Despite this prolific production, genome mining approaches have revealed that *Streptomyces* and other microbes known to produce bioactive molecules harbour many more gene biosynthetic gene clusters than earlier expected and potentially conceal new sources for novel compounds that remain cryptic in lab conditions.

Objectives: Because they mimic natural biotic interactions that can induce bioactive molecule biosynthesis, our approach is to perform co-cultures between fungi and *Streptomyces* in order to awake cryptic gene clusters in one or both partners. After that, metabolites and biosynthetic pathways will be identified by metabolomics and transcriptomics methodologies.

Methods: Fungi and environmental *Streptomyces* have been co-cultivated on two media. Metabolites extraction was performed in ethyl acetate. The antibiotic activity resulting from the interaction will be screened against indicator strains and were characterized by analytical chemistry methods (LC-DAD, GC-MS).

Results: 144 co-cultures were achieved between 9 fungi and 8 environmental *Streptomyces* on two media. Preliminary results enabled to identify 3 bacterial and fungal couples on one medium with potentially increased antimicrobial activities against Gram+ bacteria. Metabolic profiling with LC-DAD and GC-MS has revealed new metabolite production.

PW016 A model of closed equilibrium systems H20-CO2-CaCO3-NaH2PO4

Oleg Artemchuk[1]

[1]*Chemistry, Environmental Chemistry, Tartu, Estonia*

Background: The aqueous systems containing carbonaceous and phosphorous species are present in many waste waters of various types and origin. Limestone bedrock, which consists mainly of CaCO3, affects waste- and reject water treatment.

Objectives: The developed model of the equilibrium closed system H2O–(CO2)W0–CaCO3–NaH2PO4 can be used to determine concentrations of all components in the water phase over a wide range of natural and industrial conditions.

Methods: The distribution of ions and molecules in the closed system H2O–(CO2)W–CaCO3–NaH2PO4 is described in terms of a structural scheme. It have been done experimental methods as well.

Results: The model allows one to assess the impact of anthropogenic processes on the environment and could be applied by environmental technologists in water and wastewater treatment.

PW017 Bacterial internalization, a new paradigm in the interaction of mutualistic bacteria with the host

Diana Ramirez-Sanchez[1], Noemi Navarro-Lleo[2], Christine Bäuerl[2], Jose Maria Coll-Marques[2], Samuel Campista Leon[1], Gaspar Perez Martinez[2]

[1]Faculty of Biology, Culiacán Rosales, Mexico
[2]Instituto de Agroquimica y Tecnologia de Alimentos, CSIC (Spanish National Research Council) , Biotechnology, Paterna, Spain

Background: Commensal bacteria are internalized and translocated through the intestinal epithelium under pathological conditions, but recent data suggest that it could also be a more common event. While internalization was successfully demonstrated using engineered lactic acid bacteria, fundamental questions over the nature of this process remain uncertain.

Objectives: The aim of this work was to study the internalization of *Lactobacillus casei* BL23 y *Lactobacillus rhamnosus* GG in IEC (Intestinal-Epithelial-Cells) and to characterize the entry mechanisms and other information related to this interaction.

Methods: Different IEC lines (Caco-2, T84) were used to study the influence of signaling factors (IFN-γ, EGF) and specific inhibitors (EIPA, rapamycin, cytochalasin D). Then, bacterial survival was determined with gentamycin protection assays. Bacteria expressed the red and green fluorescent proteins for flow cytometry, epifluorescence and confocal microscopy experiments, also for immunohistochemistry colocalization of bacteria with clathrin and caveolin.

Results: The internalization frequency was significantly associated to the bacterial strain and the IEC line. Both probiotics were internalized through endocytosis associated to autophagy. Immune colocalization confirmed that the entry occurred by endocytic mechanisms partially dependent of clathrin and caveolin. EGF had some stimulatory effect, which supported the observation that internalization depended on the degree of confluence and differentiation rate of the IECs. Non-paracellular transcytosis in Caco-2 was observed, as well as the survival of these lactobacilli probably inside the epithelial cells for as long as 72 hours with no damage to the cells. These results will extend established concepts that explain mutualistic bacteria interactions with the host.

PW018 The first view on diversity and metabolism of microbial communities of Tuvinian hydrothermal fields (Eastern Siberia).

Stepan Toshchakov[1], Sergey Gavrilov[1], Alexander Lebedinsky[1], Aleksei Korzhenkov[2], Evgenii Lunev[3], Ilya Kublanov[1]

[1]Winogradsky Institute of Microbiology, FRC "Biotechnology" RAS, Extremophiles Metabolism Laboratory, Moscow, Russian Federation
[2]National Research Center "Kurchatov Institute", Bioinformatics, genomics and genome editing laboratory, Moscow, Russian Federation
[3]Immanuel Kant Baltic Federal University, Microbial genomics lab, Kaliningrad, Russian Federation

Background: Easternmost hydrothermal manifestations of the Baikal Rift Zone, located on the East-Tuvinian upland, represent a large geothermal field, unexplored in the context of microbial diversity. In 2015, an expedition was organized to perform field studies of microbial communities proliferating in this extreme environment.

Objectives: To characterize thermophilic microbial communities of the East-Tuvinian upland using high-throughput metagenomic approach.

Methods: Microbial communities of several hot springs with temperatures 45-83°C and pH range of 9,1-9,9 were analyzed by high-throughput sequencing of V3-V4 16S rRNA gene metabarcoding libraries. One of the communities with predominance of uncultured phylum 'Acetothermia' was chosen for shotgun metagenomic analysis. Obtained metagenomic contigs were assigned to corresponding taxa by several binning algorithms.

Results: Dominant prokariotic taxa of previously unexplored Tuvinian hydrotherms included *Anaerolineae* and *Nitrospirae*. *Anaerolineae* included anaerobic organotrophs of *Roseiflexus* genus, and uncultured group SBR1031 firstly described as a member of anaerobic cellulolytic consortia. Accordingly, analysis of metagenomic contigs revealed 202 putative glycoside hydrolases, most of which belonged to *Chloroflexi*. *Nitrospirae* were mostly represented by the thermophilic sulfate-reducer *Thermodesulfovibrio*. According to both metabarcoding and shotgun metagenomics data, uncultured group "Acetothermia" OP1 constituted 20-30% of microbial community, which probably reflects the supposed autotrophy of OP1 representatives (Takami et al., 2012). Analysis of full-length 16S rRNA gene showed that it has 83% identity with "*Acetothermus autotrophicum*" and 89% identity with recently proposed OP1-related genus "*Bipolaricaulis*" (Hao et al., 2018), supposing that Tuvinian OP1 lineage represents a new deep branch of candidate phylum "Acetothermia".

PW019 To assemble or not to resemble - bench-marking metatranscriptomic practices to for an improved and precise identification and quantification of microbial response in warming Arctic

Muhammad Zohaib Anwar[1], Anders Lanzen[2;3], Toke Bang-Andreasen[1;4], Carsten Suhr Jacobsen[1]

[1]*Aarhus University, Denmark, Department of Environmental Sciences, Roskilde, Denmark*
[2]*IKERBASQUE, Basque Foundation for Science, Bilbao, Spain*
[3]*AZTI, Pasaia, Spain*
[4]*University of Copenhagen, Department of Biology, Copenhagen, Denmark*

Background: Metatranscriptomics has been used widely for investigation and quantification of microbial communities' activity in response to external stimuli. By assessing the genes expressed metatranscriptomics provides an understanding of the interactions between different major functional guilds and the environment especially in understanding the response of microbial communities to change in Climate in Polar and Alpine regions.

Objectives: To benchmark and develop a standardized comparative metatranscriptomics workflow to analyze Arctic metatranscriptomes with increased precision and depth.

Methods: Metatranscriptomics typically utilize short sequence reads, which can either be directly aligned to external reference databases ("assembly-free approach") or first assembled into contigs before alignment ("assembly-based approach"). We compared workflows representing both alternatives, using simulated and Arctic metatranscriptomes and evaluate their accuracy in precision and recall using generic and specialized hierarchical protein databases.

Results: We show that the assembly-based approach provides significantly fewer false positives resulting in more precise identification and quantification of functional genes in metatranscriptomes. Using the comprehensive database M5nr, the assembly-based approach identifies genes with only 0.6% false positives at thresholds ranging from inclusive to stringent compared to assembly-free approach (3.6 to 15% false positives). Using specialized databases (Carbohydrate Active-enzyme and Nitrogen Cycle) the assembly-based approach identifies and quantifies genes with 3-5x less false positives. Based on this benchmarking we present a standardized and optimized workflow for identifying functional genes from Arctic metatranscriptomes. By virtue of the extensive enchmarking we also present the open source metatranscriptomics analysis workflow Comparative Metatranscriptomics Workflow CoMW which provides higher precision and minimizes false positives.

PW020 Evolution of anzymatic activities associated to carbon cycle in compost samples: beta-glucosidase, amylase, cellulase and xylanase

Mª José Estrella González[1], Francisca Suárez-Estrella[1], Mª José López López[1], Macarena del mar Jurado Rodríguez[1], Juan ANtonio López González[1], Joaquin Moreno Casco[1]

[1]*University of Almeria, Biology and Geology, Almería, Spain*

Background: Composting is defined as an eco-friendly process of biotransformation of organic waste generated by anthropogenic activities. The nutrients provided by different raw materials, as well as physical-chemical and environmental conditions of the compost pile, provide an ideal habitat for the existence of a broad microbial biodiversity. The process can be divided into four phases with respect to the temperature reached within the composting pile: mesophilic, thermophilic, cooling and maturation phases.

Objectives: The main objective of this study was to investigate the β-glucosidase, amylase, cellulase and xylanolytic activities during the different phases that take place in the composting of different waste materials.

Methods: In this work, 270 compost samples from 15 companies dedicated to the composting of vegetable waste (VR), sewage sludge (SS), urban solid waste (USR), agrifoodwaste (AW) and "alpeorujo" (ALP) were analyzed. The studies carried out were based on the follow-up of the enzymatic activities in the different thermal phases that define the composting process. These activities were also analyzed in the initial mixtures, as well as in the final products obtained in each case.

Results: The enzymatic activities indicated decreased considerably as the composting process progressed. When analyzing the results in a global way, higher values of all the activities were detected during the bio-oxidative phase of the process, given the greater amount of substrate available at the beginning of the process. In view of the results obtained, the β-glucosidase activity could be considered an adequate parameter to test the optimal evolution of the composting process.

PW021 Antimicrobial resistance in soil microbes mediated by resistance evolution and horizontal gene transfer (HGT)

Selina Bibiana Inocencia Schmidt[1], Frank Schreiber[1]

[1]*Federal Institute for Materials Research and Testing, Department of Materials and Environment, Berlin, Germany*

Background: Biocides are in contact with soils through direct application and passive leaching from protected materials. Consequently, soil microorganisms are exposed to toxic biocides even though they are not their primary targets. Soil is a large reservoir of microbial diversity and has been hypothesized to be a crucial factor for the evolution and spread of antimicrobial resistance.

Objectives: Currently, there is little knowledge on how biocides used to protect materials affect the evolution and spread of resistance. Thus, our aim is to investigate the risk for the evolution of biocide resistance and cross-resistance to antibiotics. In addition, we aim to elucidate the affect of biocides on the spread of resistance via horizontal gene transfer (HGT).

Methods: In laboratory evolution experiments we culture selected model soil microorganism with representative biocides followed by antibiotic cross-resistance determination and genome sequencing. Moreover, we investigate if the selected biocides affect the HGT frequency of plasmids that carry resistance genes among soil microorganism and the consequences for survival of the affected populations.

Results: Our initial results show only small increases of biocide resistance during serial transfers in the presence of biocides. One reason for this might be the narrow selective window for biocide resistance due to steep dose-response relationships. Furthermore, we will present results from ongoing experiments on the effects of material preservatives on HGT frequencies facilitating microbial community adaptation to stress. The results will enable future risk assessment regarding resistance evolution for biocides used as material preservatives.

PW022 SqueezeMeta, a highly portable, fully automatic metagenomic analysis pipeline for in-situ analysis of nanopore sequencing

Javier Tamames[1], Fernando Puente-Sánchez[1]

[1]*Centro Nacional de Biotecnología, Systems Biology, Madrid, Spain*

Background: The improvement of sequencing technologies has allowed the generalization of metagenomic sequencing, becoming a standard procedure for analyzing the structure and functionality of microbiomes. The bioinformatic analysis of metagenomics poses a challenge because it involves many different complex steps.

Objectives: SqueezeMeta is a full automatic pipeline for metagenomics/metatranscriptomics, covering all steps of the analysis. One of our motivations was producing a software capable to run in small desktop computers, thus being amenable to all users and all settings.

Methods: SqueezeMeta includes multi-metagenome support allowing the co-assembly of related metagenomes and the retrieval of individual genomes via binning procedures. It features several unique characteristics: Co-assembly of unlimited number of metagenomes via merging of individual assembled metagenomes, both with read mapping for estimation of the abundances of genes in each metagenome. It also includes binning and bin checking for retrieving individual genomes. Internal checks for the assembly and binning steps inform about the consistency of contigs and bins. Also, the results are stored in a mySQL database to be inspected anywhere using a flexible web interface allowing the easy creation of complex queries.

Results: We illustrate the potential of SqueezeMeta by analyzing 32 gut metagenomes in a fully automatic way, allowing to retrieve several millions of genes and several hundreds of genomic bins. We were also able to co-assemble two of these metagenomes and complete the full analysis in few hours using a simple laptop computer. It is therefore adequate for in-situ, real time analysis of metagenomes produced by nanopore sequencing.

PW023 Widening the view with TaqMan multiplex qPCR methods: Detection and quantification of antibiotic resistance genes along the wastewater treatment process

Micaela Oliveira[1;2], Mónica Nunes[1], Teresa Barreto Crespo[1;2], Ana Silva[3]

[1]*IBET - Institute of Experimental Biology and Technology, Food and Health Unit - Food Safety and Microbiology Lab, Oeiras, Portugal*
[2]*ITQB Nova, Microbiology of Man-made Environments Lab, Oeiras, Portugal*
[3]*Copenhagen University, Department of Biology - Section of Microbiology, København, Denmark*

Background: Water scarcity is a worldwide problem and agriculture alone accounts for 70% consumption of the available fresh water on Earth. This scenario makes wastewater reuse for agriculture irrigation a valuable and sustainable alternative. However, the safety of this practice is not yet assured, since the conventionally applied treatments in the wastewater treatment plants appear to be inefficient towards the removal of some emerging pollutants, such as antibiotics, antibiotic resistant bacteria and antibiotic resistance genes.

Objectives: To accurately assess and quantify the presence of resistance genes towards carbapenems, a group of last-line β-lactam antibiotics, and quinolones, a group of widely used antibiotics, in different wastewater samples, using optimized *TaqMan* multiplex qPCR protocols.

Methods: One duplex and two triplex *TaqMan* qPCR protocols were developed and optimized for the identification and quantification of five carbapenem resistance genes (duplex: *bla*KPC and *bla*OXA-48 genes; triplex: *bla*NDM, *bla*IMP and *bla*VIM genes) and three quinolone resistance genes (triplex: *qnr*A, *qnr*B and *qnr*S genes) at different points of two wastewater treatment trains.

Results: The *TaqMan* multiplex qPCR protocols were optimized with efficiencies between 90% and 110% and detection limits of 10 genome copies/µL. These qPCR protocols are now being applied to different wastewater samples, to assess the efficiency of the conventional wastewater treatments on the removal of these antibiotic related pollutants and, ultimately, to help clarifying the role of the wastewater treatment plants on the antibiotic resistance proliferation.

PW024 Distribution of glycoside hydrolases among halophilic bacteria and archaea

Johannes Werner[1]

[1]*Leibniz Institute for Baltic Sea Research, Department of Biological Oceanography, Rostock, Germany*

Background: Glycosyl hydrolases (GHs) comprise a widespread group of proteins which hydrolyse the glycosidic bond between one or more carbohydrates. Due to their protein functions, GHs have an ubiquitous role in many biological systems. GHs have been found to be prominent among halophilic microorganisms isolated from the deep sea.

Objectives: The distribution of GHs among halophiles is investigated in order to decipher the role of these enzymes in deep-sea hypersaline environments.

Methods: Machine learning and multivariate statistical analysis is used to analyse the potential separation between halophilic and mesophilic microorganisms and the distribution of different GH classes. Phylogenetic analysis is applied to determine sequence differences and provide traits about the evolutionary origin.

Results: Preliminary analyses revealed a distinct separation between halophiles and mesophiles. Interestingly, among the halophilic *Archaea* isolated from the deep sea, high numbers of GHs have been found: in particular, *Halorhabdus* spp. carry a high number of GHs. These enzymes are widely distributed over several GH families, in particular GH5, GH13, and GH51. Such an analysis provides insights into the distribution and the adaptation mechanisms of GHs in halophiles and might even aid to resolve the evolutionary origin of certain enzymes.

PW025 Bacterial antagonism against E.coliO157:H7 on leafy green vegetables

Maria Karlsson[1]

[1]*Swedish University of agriculture science, Biosystem and Technology, Alnarp, Sweden*

Background: Fruit and vegetables are an essential component of a healthy diet. During the last decades, outbreaks of foodborne illnesses have increasingly been linked to fresh and/or minimally processed fruit and vegetables. Enterohemorrhagic *Escherichia coli* causing EHEC infections, was the causal agent for major outbreaks in Europe with leafy green vegetables and sprouts. To improve food safety, microbial antagonism has received attention during recent years and could be one solution to prevent contamination of food borne pathogens on fresh produces. There are different factors to take into count when decide which antagonist is suitable for a certain purpose. The antagonist must have same nutrition preferences as the undesired microbe and have faster growth rate. It must fit into the existent microbial aggregate on the leaf surface. The antagonist also need to produce some antibacterial metabolite against the intruder pathogen

Objectives: In this study we have investigated antagonistic effect on EHEC using bacterial antagonists

Methods: First a laboratory assay was performed to optimize growth conditions. A greenhouse trial was conducted in two different experiments Experiment I).Bacterial antagonist where inoculated onto the leaves. II.) Spinach seeds were inoculated with bacterial antagonists. *E. coli* O157:H7 *gfp+* was then inoculated onto the leaves. After 24h bacteria was washed off the leaf surface and analysed by viable count.

Results: There was only a slightly reduction of EHEC when antagonists were inoculated on the leaf surface. When antagonits were inocultated on the seeds before sowing we observed a significant reduction of EHEC on the leaf surface.

PW026 The Influence of Shock Loading To Thiocyanate Biodegradation in The Nitrogen Removal System

Julia Cheng[1]

[1]*China Steel Corporation, New material R&D, Kaohsiung, Taiwan*

Background: Coke and Coke oven gas are the main fuels, raw materials for the steelmaking plant, and produced by coke making plant. Wastewater generated from coking process contains phenolics, thiocyanate, ammonia, and cyanide nearly 800, 600, 150, and 10 mg/L. In order to remove these toxic compounds and meet the industrial effluent standard of ammonia, a high efficiency biological treatment system has been developed.

Objectives: This bio-treatment system can remove >99 % phenolics, thiocyanate, and ammonia in 32 hours. After bio-treatment, the concentration of phenolics, thiocyanate and ammonia in the wastewater has to decrease to <1 mg/L.

Methods: Compared to other coking wastewater bio-treatment systems which hydraulic retention time is 3 to 14 days, the 32 h bio-system needs to be carefully taken care of by the microbiological point of view when the influent water quality fluctuates. Therefore, the microbial consortia are monitored with t-RFLP technique to control and predict the stability of pollutant removal capacity.

Results: After the bio-system has been run for 1.5 years, we have found the most essential bacteria in the bio-system to be thiocyanate-degrading bacteria. The abundance of thiocyanate-degrading bacteria *Thiobacillus* sp. is normally ~20% monitoring with t-RFLP method. However, when the cyanide shock loading exceeded 40 mg/L, not only did the abundance of *Thiobacillus* dropped to <5 %, the bacteria also released ~5 mg/L cyanide and ~20 mg/L ammonia after thiocyanate degradation, delaying the ammonia oxidization. The relativity of the contaminants removal efficiency stability and the abundance of thiocyanate-degrading bacteria is still under investigation.

PW027 Bacterial microbiome of Free-Living Amoeba (FLA) isolated from wastewater

Laura Moreno-Mesonero[1], María Antonía Ferrús[2], Yolanda Moreno Trigos[1]

[1]*Research Institute of Water and Environmental Engineering, Valencia, Spain*
[2]*, Biotechnology, Valencia, Spain*

Background: Free-living amoebae are ubiquitous protozoa frequently found in water. It has been shown that they can harbour bacteria inside them, thus protecting them against unfavourable environmental conditions. The most widely studied relationship of FLA is with *Legionella* although there are interaction examples of many other pathogenic bacteria. Currently, there are very few studies which determine bacterial microbiome of FLA isolated from water sources.

Objectives: The objective of this work was to determine the bacterial microbiome of FLA isolated from wastewater.

Methods: Eleven wastewater samples, before and after tertiary treatment, were processed. Five litres of each sample were filtered through 3µm membranes and incubated on non-nutrient agar. Only if FLA were isolated, plate's content were collected and treated with 100ppm of sodium hypochlorite. Then, PMA treatment was performed and DNA was extracted to be used in Illumina MiSeq 16S amplicon-based metagenomics employing 341F and 802R primers.

Results: A total of 691,316 sequences were obtained after sequencing. After applying quality filters and removing chimeras, 547,664 sequences remained, which were grouped into 12,281 OTUs. The most abundant Phyla among all samples were Proteobacteria, Plactomycetes, Bacteroidetes and Firmicutes, which accounted for 81.93% of the total population. The most abundant genes were *Bacillus*, *Aeromonas*, *Flavobacterium*, *Isosphaera* and *Sphingobium*, which accounted for 36.06% of the total population. Among all detected genes, different pathogenic protozoa such as *Aeromonas*, *Helicobacter*, *Legionella*, *Mycobacterium* or *Pseudomonas* were detected as part of wastewater FLA microbiome.

This study has been supported by by the Generalitat Valenciana through project the AICO/2018/273

PW028 Agronomic practices affect the equilibrium of soil microbiota composition and function in sandy corn fields

Huaying Zhang[1], Jinghui Liu[1], Devon Radford[2], Wen Chen[2]

[1]*Inner Mongolia Agricultural University, College of Agronomy, Hohhot, China*
[2]*Agriculture and Agri-Food Canada, Science and Technology Branch, Ottawa, Canada*

Background: Horqin Sandy Land, located in the east of the Inner-Mongolian Plateau, is China's largest sandy land and a typical farming-pastoral region, with more than 70% arable land is used to grow maize. How soil microbiota provide ecosystem services in sandy cornfields has not been reported.

Objectives: 1) To obtain an in-depth understanding of how different agronomic practices (fertilization, tillage and soil amendment with hydrogels) impact the soil microbiota. 2) To identify microbial indicators associated with soil health/productivity.

Methods: A total of 132 bulk soil samples were collected. The diversity and community profiles of soil microbiota were assessed based on fungal ITS1 and bacterial 16S rRNA V4 metabarcodes.

Results: The species richness of the bacterial and fungi communities was 3970±371 and 1693±568, respectively; and in general, was higher at post-harvest than at the time of sowing. Compared with conventional tillage, conservation tillage (no till, mulching, subsoiling and stubble) significantly improved the diversity of the fungal but not the bacterial communities. The divergence of the fungal communities was strongly associated with soil structure and moisture content, while that of the bacterial communities was more associated with nutrient resources. The sandy cornfields were dominated by *Proteobacteria*, *Actinobacteria* and functional bacteria involved in fermentation and nitrate reduction, as well as fungal genera *Guehomyces, Alternaria* and *Mortierella*. Bioindicators and co-occurrence patterns associated with high/low-yield fields were identified. Network analyses further suggest that the equilibrium between beneficial and detrimental members of the microbiota is vital for crop and soil health and productivity.

PW029 How safe are natural pesticides: A case study on rhizospheric microbiome of Vigna radiata

Upma Singh[1], Varsha Ashok Walvekar[1], Shilpi Sharma[1]

[1]*Indian Institute of Technology Delhi, Department of Biochemical Engineering and Biotechnology, New Delhi, India*

Background: Excessive pesticide application deleteriously impacts the indigenous rhizospheric microbiome thereby reducing soil-health. Natural pesticides have been known as a safer substitute because of biological origin, however there have been reports of adverse effects of natural pesticides on soil microbiota. So, microbiome-based solution can serve as an opportunity to negate such disturbances and enhance plant productivity.

Objectives: The purpose of the study was two-fold, viz. To investigate the impact of two chemical pesticides (cypermethrin and chlorpyrifos), and a natural pesticide (azadirachtin) on rhizospheric microbial dynamics in *Vigna radiata,* and to strategically employ bioinoculants with plant growth promoting (PGP) properties to mitigate the ecological disturbances.

Methods: The rhizospheric microbial dynamics was analysed through culture-dependent and –independent approaches. Qualitative analysis was performed by 16S rRNA PCR-DGGE, and quantitative analysis was done by qPCR of various genes and transcripts (16S rRNA, *nifH, amoA, narG, nirK*). Azadirachtin-degrading strains were assessed for PGP properties and checked for azadirachtin tolerance followed by *in vitro* and *in planta* study. Azadirachtin concentration was monitored by HPLC.

Results: culture-dependent study revealed the adverse effects of pesticides on rhizospheric microbiota. Similarly, reduced abundance of 16S rRNA gene and transcripts was observed. HPLC analysis showed higher rate of azadirachtin utilization by the bioinoculants in pesticide-supplemented culture. Natural pesticides had effects similar to that of chemical pesticides on rhizospheric microbiome. Application of bioinoculants equipped with pesticide-degrading ability could reduce the damaging effects of pesticide and would serve as a biologically safe, eco-friendly and sustainable alternative to chemicals in current agriculture practices.

PW030 Incidence, abundance and characterization of ndm variants in a city waste polluted river from northern west bengal, india

Shriparna Mukherjee[1], vivek ranjan[2], ranadhir chakraborty[2]

[1]Prasannadeb Women's College, Botany, Jalpaiguri, India
[2]North Bengal University, Omics Laboratory, Department of Biotechnology, Siliguri, India

Background: The molecular epidemiology of *bla*-NDM1 positive Enterobacteriaceae is rapidly evolving. It is likely that identification of NDM variants may signal an ongoing and rapid evolution of this gene.

Objectives:

a. To determine the prevalence of NDM-1 in enterobacterial isolates of an anthropogenically stressed river Karala
b. To explore the diversity of encoding genes
c. To estimate the carrier state of NDM-1 producers to predict its genetic spread

Methods: Water samples, collected from three different sampling stations, were analyzed following standard microbiological methods. Selective microbiological media were used for isolation and identification of the different enterobacterial isolates. Antibiotic susceptibilities to various ß-lactam and non- ß-lactam groups of antibiotics were determined using CLSI guideline. EDTA impregnated imipenem plates prepared on LB agar were used for screening of metallo beta lactamase (MBL) producing isolates. Carba-NP test followed by PCR detection of MBL genes were employed to identify potential carabapenemase producers. The transfer of resistance was tested using conjugation studies.

Results: A large random collection of (n=2326) imipenem resistant bacterial isolates were isolated from the water samples. 879 (37.79%) colonies were identified primarily as MBL producers. Of these, 20.47% (180) of the isolates were found positive for carba-NP test and were examined for the presence of MBL genes. Nine isolates produced amplicons specific of NDM gene. One of them also produced class A beta-lactamase group enzyme (PSE-1). Conjugation was found positive with twenty two isolates. Frequencies of the conjugal transfer ranged from $9.76\times10^{-7}$to 1.37×10^{-4}. All the transconjugants were multidrug resistant in nature.

PW031 Principal component analysis reveals microbial biomass carbon as effective bioindicator of health status petroleum-polluted agricultural soil

Ncheta Orakwelu[1], Chukwudi Onwosi[2]

[1]*Nnamdi Azikiwe University, Applied Microbiology and Brewing, Awka, Nigeria*
[2]*University of Nigeria, Nsukka, Microbiology, Nsukka, Nigeria*

Background: Bioremediation has been recognized as an effective method of restoring hydrocarbon polluted soils. A number of monitoring tools has been identified as good indicators of effective bioremediation. Soil enzymatic activities are the most reported biological parameters for the assessment of soil health. Thus, other soil microbial activities are often neglected.

Objectives: Therefore, there is need to evaluate the effectiveness of other microbial activities such as microbial biomass carbon, nitrogen and respirometric index in monitoring of toxicity of petroleum hydrocarbon contamination in soil.

Methods: Sawdust was used as the biostimulant for the biodegradation of artificial diesel-polluted soil samples. Soil microbial enzymatic activities, microbial biomass carbon, nitrogen and phosphorus , respirometric index and total petroleum hydrocarbon (TPH) concentration were monitored to evaluate the efficiency of the bioremediation process. The nth-order kinetic model was used in modeling the rate of biodegradation of the hydrocarbon. Principal component analysis was deployed to select the most sensitive bioindicators.

Results: TPH content reduced from 14221 mg/kg to 270 mg/kg following a first order kinetics. The rate constants (*k*) for TPH removal were $4.417d^{-1}$ and $0.2670d^{-1}$ for sawdust-amended and unamended soil, respectively. This implied that, sawdust amendment resulted in degradation rate 16.5 times faster than unamended soil. Microbial biomass carbon, catalase, lipase and dehydrogenase were observed as the most responsive bioparameters. A positive relationship between TPH removal and the four most sensitive bioparameters suggests that they could be effectively used as ecomonitoring tools.

PW032 Deciphering the alteration in river bacterial communities during the mass bathing event and its impact on public health

Kunal Jani[1;2], Jayashree Bandal[3], Vinay Rale[1], Yogesh Shouche[2], Avinash Sharma[2]

[1]Symbiosis International (Deemed University), Symbiosis School of Biological Sciences, Pune, India
[2]National Centre for Cell Science, National Centre for Microbial Resource, Pune, India
[3]KTHM College, Department of Microbiology, Nashik, India

Background: The Kumbh Mela represents largest religious congregation on the earth. It is hosted every 12 years by 4 Indian cities in rotation. The popularity of the event drew as large as 120 million pilgrims during 2013, to have a holy dip in the river. Considering the virtue of huge population density incorporating enormous load of microbiota during bathing event act as a critical biotic strain, affecting microbial community and accompanying environmental factors. Assemblage of such contaminants in river during the event increases risk of water-borne infections and diseases which may have serious public health implications.

Objectives: Analzying bacterial communities and associated functional attributes under spatiotemporal constrain of Kumbh Mela.

Methods: Assessment of bacterial community structure using targeted amplicon sequencing. Characterization of source of microbial contaminants and its potential role in infection and diseases using imputed metagenomics.

Results: Molecular assessment of bacterial community across the mass bathing event indicated loss of phylum Actinobacteria, Bacteroidetes and Proteobacteria with specific augmentation of phylum Firmicutes. Further, the RT-PCR estimates depicted ~130 increase bacterial load suggesting bathers being source of non-point pollution. Additionally, river bacterial communities depicted negative correlation with the event casting substantial (~37.5%) loss of diversity. Bayesian mixing model allowed characterizing sourced microbial contaminants of skin (~2.32x) and fecal (~2.89x) origin during Kumbh Mela. Imputed metagenome demonstrated the increase in drug resistance and inflated pathogenic microorganisms during the event. Our study provides a first report addressing impact of Kumbh Mela on river bacterial communities and associated potential risk of infectious diseases outbreak.

PW033 Biogeochemical effects of temperature changes on microbial communities and groundwater contaminations

Mohammad Sufian Bin Hudari[1], Carsten Vogt[1], Hans-Hermann Richnow [1]

[1]Helmholtz Centre for Environmental Research - UFZ, Department of Isotope Biogeochemistry, Germany

Background: Aquifer thermal energy storage (ATES) is an important source of renewable energy. These are usually operated at temperatures between 5°C and 20°C, but temperatures of above 50°C are becoming increasingly commonplace. In normal ambient conditions, contaminants can be removed by natural attenuation under aerobic or anaerobic conditions by indigenous microorganisms. While it is believed that perturbations exceeding normal temperature variations may influence biodiversity and potential ecosystem services in the subsurface environment, their consequences remain unclear.

Objectives: We intend to better understand how temperature may affect the biota in ATES systems and pollutant bioremediation in contaminated aquifers. Findings from this study will allow us to better understand the limitations and effects of higher temperatures on population composition and its attenuation potential.

Methods: We set up microcosms comprising sediments from pristine and contaminated aquifers spiked with ^{13}C-labelled substrates (e.g., benzene, acetate) and incubate at temperatures between 10°C to 80°C. Mineralization kinetics is determined by $^{13}CO_2$ analysis, and stable-isotope probing (SIP) techniques are used to identify the responsible substrate-assimilating organisms.

Results: Preliminary results from one microcosm study with sediments from a contaminated site spiked with ^{13}C-labelled acetate under sulfate reducing conditions show that mineralization occurred at temperatures between 10°C and 80°C. However, after 36 days, analysis of $^{13}CO_2$ suggest that mineralization in setups incubated at 12°C, 45°C, 60°C, and 80°C seem to have stopped while this continues to be observed at 25°C and 37°C.

PW034 Novel sulfate- and sulfur-reducing bacteria from the Black Sea: metagenomics, enrichment and isolation

Daan M. van Vliet[1], F.A. Bastiaan von Meijenfeldt[2], Sebastiaan Versteeg[1], Robert den Breems[1], Bas Dutilh[2;3], Laura Villanueva[4], Alfons J.M. Stams[1;5], Irene Sánchez-Andrea[1]

[1]*Wageningen University, Laboratory of Microbiology, Wageningen, Netherlands*
[2]*Utrecht University, Theoretical Biology and Bioinformatics, Utrecht, Netherlands*
[3]*Radboud University Medical Centre, Centre for Molecular and Biomolecular Informatics, Nijmegen, Netherlands*
[4]*Royal Netherlands Institute for Sea Research (NIOZ) and Utrecht University, Department of Marine Microbiology and Biogeochemistry, Netherlands*
[5]*University of Minho, Centre of Biological Engineering, Braga, Portugal*

Background: Anoxic and euxinic marine waters are of importance to the biogeochemistry of modern-day ocean as well as the ancient Proterozoic ocean. In these environments, sulfate- and sulfur-reducing bacteria (SRB) play a key role through the reduction of oxidized sulfur compounds such as sulfate, thiosulfate and elemental sulfur to sulfide. In previous studies, these marine SRB were found to be comprised of both canonical deltaproteobacterial and non-canonical, mostly uncultured lineages.

Objectives: We aimed to test these findings, and to assess the diversity of SRB in the Black Sea, the largest euxinic basin on the planet and a well-studied model ecosystem.

Methods: To this end, we screened a cross-assembled metagenome and binned genomes for functional marker genes. In parallel, we set up duplicate anaerobic enrichments and dilution series selecting for the reduction of sulfate, thiosulfate or elemental sulfur with various electron donors. The enriched microorganisms were identified through 16S rRNA gene amplicon sequencing.

Results: From a cross-assembled metagenome, we retrieved 86 *dsrD* genes and genomes of seven *Deltaproteobacteria* and one *Thermodesulfovibrionia* with a complete dissimilatory sulfate reduction pathway. Moreover, we detected 46 genes encoding iron-sulfur molybdoenzymes related to the polysulfide/sulfur/thiosulfate reductase catalytic subunit, present in *Deltaproteobacteria*, *Planctomycetes* and *Marinimicrobia* binned genomes. Furthermore, we isolated and characterized a novel sulfate-reducing *Desulfobulbaceae* bacterium related to *Desulfotalea* and *Desulfopila* species, which potentially represents a novel genus. In conclusion, a combined metagenomics and anaerobic cultivation approach revealed novelty within canonical and non-canonical SRB lineages, and confirmed the sulfate- and sulfur-reducing metabolism of specific taxa.

PW035 Río Agrio: microbial diversity across an extreme environmental gradient

Francisco Issotta[1;3], Dilanaz Arisan[1], Ana Moya-Beltrán[1;3], Matías Castro[1], Ricardo Ulloa[4], Patricia Chiacharini[4], Alejandra Giaveno[4], Mayra Cuevas[5], Fernando Pedrozo[5], Pedro Temporetti[5], Alejandra Lima[6], Sofía Urbieta[6], Edgardo Donati[6], Barrie Jhonson[7], Raquel Quatrini[1;2]

[1]*Fundación Ciencia y Vida, Microbial Ecophysiology Laboratory, Santiago, Chile*
[2]*Millennium Nucleus in the Biology of the Intestinal Microbiota, Santiago, Chile*
[3]*Universidad Andres Bello, Facultad de Ciencias Biologicas, Santiago, Chile*
[4]*Universidad Nacional del Comahue, PROBIEN, Neuquén, Argentina*
[5]*Universidad Nacional del Comahue, INIBIOMA-CRUB, Bariloche, Argentina*
[6]*Universidad Nacional de La Plata, CINDEFI, Buenos Aires, Argentina*
[7]*Bangor University, School of Natural Sciences, Bangor, United Kingdom*

Background: The Copahue-Caviahue-ChanchoCo geothermal (CCCG) system is a natural extreme environment located in the Andes mountain. Two hydrothermal springs near the volcano crater are the source of an acidic watershed of unique characteristics in South America named Río Agrio (RA). Along its journey from the source to Lake Caviahue and downstream, this river features an ample gradient of pH (pH 0.5–8.5), temperature (10-80ºC) and conductivity (0.5-500 mS/cm). Previous studies have shown that the CCCG system host a rich diversity of acidophilic prokaryotes, including representatives of the acidithiobacilli. However, little is know about the variability and distribution patterns of the dominant taxa.

Objectives: Directed metagenomics (DMG) of the RA gradient was used to assess the microbial distribution, abundance and diversity. These results were analyzed in the context of the hydrogeochemical features of the sampling points.

Methods: Microbial biomass was recovered across the basin of the river for total DNA extraction, PCR and amplicon sequencing. Samples were collected at points were variations in pH, temperature and conductivity could be registered. Selective isolation of acidithiobacilli was pursued for additional marker gene sequencing.

Results: Different niches were distinguished along the gradient. DMG data generated provided new insights into the community structure in these niches and the composition and diversity changes occurring along the river. The occurrence and distribution patterns of several microbial acidophiles (e.g. *Acidithiobacillus*) were defined, along with their microdiversity in distinct niches. This work sets the basis for further studies aiming to explore the eco-evolutionary processes that shape this ecosystem.

PW036 Living under the sun: microbial ecology and potential applications of the solar panel microbiome

Kristie Tanner[1], Esther Molina-Menor[2], Katherine B Louie[3], Suzanne M Kosina[4], Marc W. Van Goethem[4], Benjamin P. Bowen[3;4], Trent R. Northen[3;4], Juli Peretó[1;2;5], Manuel Porcar[1;2], Cristina Vilanova[1]

[1]*Darwin Bioprospecting Excellence S.L., Paterna, Spain*
[2]*I2SysBio (Institute for Integrative Systems Biology), Paterna, Spain*
[3]*Lawrence Berkeley National Laboratory, Joint Genome Institute, Berkeley, United States*
[4]*Environmental Genomics and Systems Biology Division, Lawrence Berkeley National Laboratory, Berkeley, United States*
[5]*University of Valencia, Department of Biochemistry and Molecular Biology, Burjassot, Spain*

Background: Microorganisms are ubiquitous and can be found in a wide range of extreme and unusual environments, including solar panels, that harbor a diverse microbial community similar to other highly irradiated environments (i.e. plant-surfaces and deserts) and are composed mainly of desiccation/irradiation-adapted microorganisms. Solar panels represent a particularly interesting environment due to (1) their worldwide standard structure and orientation, representing a proxy of sun-exposed natural environments, and (2) the presence of pigment-producing bacteria with potential applications in the food, cosmetic and pharmacological industries.

Objectives: The objective of this study is to shed light on the ecology and potential applications of the solar panel microbiome by comparing the taxonomic and functional microbial profiles on panels from distant geographical locations: Berkeley (USA), Valencia (Spain), Tromsø (Norway) and South Shetlands (Antarctica).

Methods: To accomplish this objective, standard microbiological culturing methods have been combined with culture-independent analyses (metagenomic sequencing and metabolomics).

Results: Solar panels proved to be very rich in cultivable bacteria, including a large fraction of pigmented colonies able to grow between 4 and 37 °C, to resist desiccation and UV-light irradiation and with glass-colonization abilities. Taxonomic analyses revealed a core of shared microbial taxa in all locations, including *Sphingomonas*, *Pseudomonas*, *Streptomyces*, *Methylobacterium* and, especially, *Hymenobacter*. Functional and metabolomic analysis revealed pathways involved in the survival of microorganisms on solar panels (i.e. capsule development, metabolite repair, carotenoid biosynthesis), whereas genes involved in photosynthetic pathways and general autotrophic subsystems were rare, suggesting that these pathways are not critical for persistence on solar panels.

PW037 an investigation into the drivers that impact faecal indicator organisms (fio) concentrations which vary over a tidal cycle in fingal, county dublin.

Jayne Stephens[1], Laura Sala-Comorera[1], Liam Reynolds[1], Niamh Martin[1], Aurora Gitto[1], Tristan Nolan[1], brian reynolds[2], yvonne cannon[2], Wim Meijer[1]

[1]*university college dublin, school of biomolecular and biomedical science, Ireland*
[2]*fingal county council, water, Ireland*

Background: Bathing water quality is monitored all year around to ensure the health and safety of the public according to the EU Bathing Water Directive 2006/7/EC. To determine bathing water quality samples are taken on a predetermined day at compliance points on beaches throughout Europe.

Previous studies in both Wales and Ireland (SmartCoasts Project) have shown that levels of FIO vary throughout a day and can be impacted by various events or conditions. This highlights that taking a single sample at one time point may not be sufficient to determine water quality.

Objectives: The present study was carried out twice on both a 'poor' categorized bathing water Portrane beach and an 'excellent' Donabate beach during the bathing season to identify the main drivers that impact FIO levels in bathing waters in Ireland.

Methods: Transect studies were carried out over a 12 hour tidal cycle, involving half-hourly sampling. Concentrations of *E. coli*, intestinal enterococci, somatic coliphages and *clostridium perfringens* were determined. Physiochemical parameters – dissolved oxygen, salinity, pH, sea temperature, total dissolved solids and turbidity were also taken. Environmental parameters that might impact FIO variations were then examined[1]. Hydrometric – rainfall, river and stream discharge, tidal cycle. Meteorological – sunshine levels, temperature, wind speed/direction.

Results: Our current data indicate that FIO levels vary by 1 logarithm, indicating bathing water represents a complex environment and to fully understand FIO levels in bathing waters drivers are identified. Results indicate that there is a tidal impact on these FIO levels on Portrane bathing water.

PW038 Biodiversity and metabolic potentials in deep quartzitic caves

Daniele Ghezzi[1], Francesco Sauro[2;3], Andrea Columbu[2], Pei-Ying Hong[4], Jo De Waele[2], Davide Zannoni[1], Martina Cappelletti[1]

[1]*Alma Mater Studiorum - Università di Bologna, Department of Pharmacy and Biotechnology, Bologna, Italy*
[2]*Università degli studi di Bologna - Sede di Mineralogia, Bologna, Italy*
[3]*European Space Agency*
[4]*King Abdullah University of Science and Technology, Thuwal, Saudi Arabia*

Background: Subsurface ecosystems are largely unexplored habitats where microbial life depends on the interaction with minerals and limited organic sources. In this respect, deep caves might function as long-term reservoirs of still undescribed microorganisms and can be used as model systems to explore the origin and evolution of life on Earth and on other planets. In this work, we investigated the microbial communities inhabiting one of the longest and oldest quartzitic caves, Imawarì Yeuta, located on venezuelan tepui. This cave has unique scientific potentials in relation to its great age (estimated over 30 million years), the complete absence of anthropic activities, and the presence of unique silica-based biospeleothems (patinas, deposits) which have never been described before in such amount and diversity.

Objectives: The aim of this study is to define the microbial populations associated with various biodeposits from different niches in Imawarì Yeuta Cave, with the purpose to explore peculiar microbial processes involved in microbial adaptation and evolution within oligotrophic and remote environments with astrobiological relevance.

Methods: The full-length 16S rRNA genes and the complete metagenomes were analysed in order to describe both the phylogeny and the functions of the microbial communities.

Results: The biospeleothems collected from Imawarì Yeuta Cave hosted diverse and peculiar microbial community structures. Novel Chloroflexi and Actinobacteria were found in quartz-rich biospeleothems, while specific chemolithotrophic Alphaproteobacteria populated amorphous silica-based mats. Peculiar metabolic features were detected associated to each biospeleothem revealing specific microbial interactions and influence on the cave system minerals and rocks.

PW039 Comparison of the plant growth promotion performance of a consortium of Bacilli inoculated as endospore or as vegetative cells

Isha Ihtsham Hashmi[1], Christophe Paul[1], Andrej Al-Dourobi[1], Frederic Sandoz[1], Pricilla Deschamps[1], Thomas Junier[2], Pilar Junier[1], Saskia Bindschedler[1]

[1]*Laboratory of microbiology, University of Neuchâtel, Neuchâtel, Switzerland*
[2]*Vital-IT group, Swiss Institute of Bioinformatics, Lausanne, Switzerland*

Background: In conventional agriculture, inappropriate management practices and use of agrochemicals has led to major impacts on both soil and water. Thus, the search for sustainable alternatives in agriculture has become a priority. Endospore forming bacteria are well-known to enhance plant growth through biocontrol and biofertilization activities. Also their ability to withstand harsh conditions make them good candidates as a potential solution for bio-inoculation.

Objectives: To investigate the effectiveness of three *Bacillus* strains with plant growth promoting abilities to promote oat (*Avena sativa)* growth, either as individual inocula or as a consortium and to assess their impact on the native soil microbial communities.

Methods: Three *Bacillus* strains were used for bio-inoculation in pots (with sterile and non-sterile soil) and in the field. Oat seeds were coated with bacteria applied either as vegetative cells or endospores. Bacteria were applied individually or as mixed consortia.

Results: Bio-inoculation had a positive effect on plant growth and fitness. Consortia showed more robust performances in soil than individual inocula. In the field, the consortium of vegetative cells was more efficient than the consortium of endospores. Analysis of the microbial communities of bulk and rhizospheric soils, as well as the roots, demonstrated that the inoculum successfully colonized the rhizosphere, without modifying the overall structure of microbial communities in soils. The direct application of the consortium on seeds favored rhizospheric colonization and resulted in a minimal impact on native bacterial communities in response to the application.

PW040 Development of multiplex PCR assay for detection of bacterial and fungal pathogens causing diseases in tomato and cucumber

Hanna Bareika[1], Illia Kazlouski[1], Leonid Valentovich[1], Anastasiya Sidarenka[1], Marina Titok[1], Emiliya Kalamiyets[1]

[1]*The Institute of Microbiology of the National Academy of Sciences of Belarus*

Background: Diseases, causing by phytopathogenic bacteria and fungi, are one of the main contributors to economic losses in agriculture. Monitoring of the infection agents using high specific, sensitive, reproducible, quick, cost effective and highthroughput diagnostic assays, is the majour strategy of agricultural stability.

Objectives: The aim of the study was to design genus- and species-specific primers and to compose multiplex PCR assay system that could afford detection of bacterial and fungi pathogens of tomato and cucumber, in affected plants, seeds, water and soil by running single reaction.

Methods: Bioinformatics, taxon-specific and multiplex PCR

Results: Original genus- and species-specific primers for detection of the most important tomato and cucumber microbial pathogenes, including fungi *Alternaria* sp.*, Fusarium* sp., *Cladosporium cladosporioides, Botrytis cinerea, Sclerotinia sclerotiorum, Plectospaerella cucumerina, Didymella bryoniae* and bacteria *Clavibacter michiganensis, Pseudomonas syringae, Pseudomonas corrugata, Pectobacterium carotovorum, Xanthomonas campestris* were designed. For simultaneous diagnostics of causing agents of such diseases as wilt, spotting and blight, particular combinations of primers were obtained and PCR protocols were optimized. Main benefits of developed multiplex PCR assay are: i) easy-to-use, sensitive and high-throughput; ii) cost-effective compared to available analogues; iii) enables diagnostics of pathogens, causing diseases with similar symptoms; iiii) early detection of infectious agents in seeds, soil and water. Application of the multiplex PCR supports elaboration of effective “target microbe” disease management strategy to prevent epiphytoty and crop yield loss, obtain safe and healthy agricultural products, which is essential for safe and sustainable farming.

PW041 Genome and comparative proteome analysis of glucose degradation in Bacillus stamsii - axenic culture versus methanogenic co-culture

Fabian Gräßle[1], David Schleheck[1], Bernhard Schink[1], Nicolai Müller[1]

[1]*University of Konstanz, Biology, Konstanz, Germany*

Background: In sediments of freshwater lakes, methanogenic communities are proven to utilize a broad spectrum of organic substrates. In Upper Lake Constance, *Bacillus stamsii* was isolated via agar shakes in the presence of the methanogenic archaeon *Methanospirillum hungatei* and found to be an abundant, obligately syntrophic glucose degrader. Later, it was further found to be capable of axenic growth when supplied with amino acids and pyruvate in addition to glucose. Whilst in co-culture with *Methanospirillum hungatei, Bacillus stamsii* excretes both hydrogen and formate for interspecies electron transfer. Thereby, the levels of theses metabolites are maintained at a sufficiently low and thermodynamically favorable standing.

Objectives: Even though B. stamsii does not grow anaerobically in axenic culture, it would be able to ferment glucose to lactate, as lactate dehydrogenase is constitutively expressed. It is therefore questionable, why *B. stamsii* obligately depends on either syntrophic cooperation or pyruvate as growth stimulating agent during anaerobic, fermentative growth. The possible reasons are discussed based on proteome analysis.

Methods: The genomic DNA of *Bacillus stamsii* was sequenced and annotated. Furthermore, the proteome of the axenic culture was compared to the one of the co-culture.

Results: The sequencing results yielded a genome size of 3 441 423 bp and 3 980 protein coding genes. Analysis of both the cytoplasmic and membrane proteome revealed, that all glycolysis genes are expressed under all growth conditions. In contrast, enzymes involved in hydrogen and formate formation were specifically upregulated in the co-culture.

PW042 Comparative genomic insights of Halothece sp. PCC 7418 in the context of its evolutionary environmental implications

Victor Fernandez[1], Carlos Enrique-Payá[2], Nona Sheila Agawin-Romualdo[1], Antoni Bennasar Figueras[1]

[1]*Balearic Island University, Biology, Palma, Spain*
[2]*Microbiology group, Department of Biology, University of the Balearic Islands, Palma de Mallorca, Spain*

Background: *Halothece* sp. PCC 7418 is a unicellular nitrogen fixing cyanobacteria associated to *Posidonia oceanica*. Originally called *Synechococcus* PCC 7418, and later reclassified as *Cyanothece*; nowadays is known as *Aphanothece halophytica* 7418 or *Halothece* sp. PCC 7418. We use this strain as a model to study how the phytoplanktonic communities in *P. oceanica* respond to changes in nutrients like P, Fe and N; the usual oligotrophic characteristics of the Mediterranean Sea in which *P. oceanica* constitutes the climax community.

Objectives: The main goals of this study are to definitively clarify the taxonomic status of this species and to characterize the genes contributing to changing levels of P, Fe and N.

Methods: Pangenome analysis was carried out applying an in house pipeline (UHGene) that consisted of identification of orthologous, gene clustering, and the estimation of the core- and pan genome. A Make Position Frequency Matrix (PFM) was constructed to search DNA motifs for PhoB, Fur and NtcA.

Results: A pangenome analysis was performed to clarify the evolutionary context of *Halothece* sp. PCC 7418. A "strict" core-genome was obtained to confirm its species status. The genes exclusive were revealed and the complete genome checked for potential genes evolved under positive selection. Finally, the in-depth analyses of *Halothece* sp. PCC 7418 genome shown genetics modules related to adaptation and survival, like CRISPR-Cas and toxin-antitoxin systems. The gene boxes for PHO, Fur and NtcA were predicted, and the shared synteny with other species used to evaluate its potential response under oligotrophic conditions.

PW043 In vitro antibiofilm effect and cytotoxicity, and in vivo toxicity of Persicaria maculosa and Bistorta officinalis ethanolic extracts

Marina Jovanović[1], Biljana Nikolić[1], Ivana Morić[2], Lidija Senerovic[2], Aleksandar Pavić[2], Emilija Svirčev[3], Dragana Mitić-Ćulafić[1]

[1]University of Belgrade-Faculty of Biology, Chair of Microbilogy, Belgrade, Serbia
[2]Institute of molecular genetics and genetic engineering, Laboratory for Microbial Molecular Genetics and Ecology, Belgrade, Serbia
[3]University of Novi Sad-Faculty of Sciences, Department of Chemistry, Biochemistry and Environmental Protection, Novi Sad, Serbia

Background: The identification of antimicrobials active against biofilm formation is very important in the field of healthcare and food processing. *Persicaria maculosa* and *Bistorta officinalis* (fam. *Polygonaceae*) produce a various active substances and have been used as edible and medicinal plants worldwide.

Objectives: The aim was to evaluate anti-biofilm and anti-quorum sensing effects of ethanol extracts of *P. maculosa* (PEM) and *B. officinalis* (BIO), and to determine the risk assessment of their use.

Methods: The extracts were chemically characterized by LC/MS/MS analysis. Following assays were applied in order to evaluate the antibacterial properties: microdilution, static biofilm formation inhibition, quorum sensing inhibition and swarming motility. Moreover, the interference with QS pathways was additionally monitored. Bacteria used were*: Chromobacterium violaceum* CV02, *Pseudomonas aeruginosa* strains PAO1 (DSMZ 22644), PAO1 Δ*pqsA* (CTX *lux*::*pqsA*), PAOJP2/pKD-rhlA (ΔrhlA P*rhlA::lux*), PA14 and PA14-R3 (ΔlasI P*rsal::lux*). The cytotoxic effect was tested by MTT assay on normal fetal fibroblasts, while toxicity was assessed in zebrafish model.

Results: PEM and BIO were rich in phenolics. They inhibited or stimulated the biofilm formation, dependent on the applied concentrations and bacterial genetic backgrounds. Further, they affect the virulence characteristics: swarming motility and the production of violacein and pyocyanin. These effects were realized through the partial suppression of LasR and RhlR receptors and the interference with PQS signaling pathway. The antibiofilm active concentrations were neither cytotoxic nor toxic. The results confirm the health benefit of extracts and justify the use of *Persicaria maculosa* and *Bistorta officinalis* as edible and ethnopharmacological plants.

PW044 Evaluation of biodegradation of atrazine herbicide in contaminated soils using biostimulating agents and indigenous microbiota

Godwin Aliyu[1], Chukwudi Onwosi[1]

[1]*University of Nigeria, Nsukka, Nsukka, Nigeria*

Background: The continuous use of herbicides such as atrazine in agriculture has resulted in different environmental hazards. This is becuase, apart from weedkilling, these chemicals supress the soil biological and physicochemical components, and hence impair the effective nutrient cycling in the environment. Hence, there is need to find ways to amend the soil to mitigate the toxic side effects of the chemicals.

Objectives: To compare the effectiveness of biochar, poultry manure and autochthonous microbiota towards atrazine degradation in soils. Also, the extent to which these treatments improved the soil physicochemical and the biological properties should be monitored.

Methods: Different treatment units were set up for amendment of the atrazine-contaminated soils with biochar and poultry manure. Atrazine-degrading bacteria isolated from an agricultural field were used in bioaugmentation. Total viable cell count, physicochemical properties and the residual atrazine determination were monitored. The degradation of atrazine was modelled using the nth order kinetic algorithm

Results: The isolated bacterium was identified as *Alcaligenes faecalis*. Atrazine degradation was highest in the soil amended with manure alone (99.95 %) at the fifth week, while soils amended with biochar alone and biochar/manure had 98.04 % and 98.39 % degradation, respectively. The soil amended with *Alcaligenes faecali*s had a 71.29 % atrazine degradation. The degradation of atrazine in three treatment units (soil + biochar, soil + manure, and soil + biochar + manure) fit adequately the second order kinetics while the treatment with soil *A. faecalis* adequately fit the first order degradation rate.

PW045 Riverine microbial communities respond to chronic contamination

Errol D. Cason[1], Alba Gómez[1], Angel Valverde[1]

[1] *Microbial, biochemical and food biotechnology, Bloemfontein, South Africa*

Background: Human activities such as agriculture and mining are leading causes of water pollution worldwide. Individual contaminants are known to negatively affect microbial communities. However, the effect of multifaceted pollution on these communities is less well understood.

Objectives: To fill this gap, we investigated the effects of multisource chronic pollution (derived from human settlements, agriculture and mining activities) on microbial community (bacterial and archaeal) composition and potential function in water and sediments from the Olifants River catchment, Mpumalanga and Limpopo Provinces, South Africa.

Methods: The links between multisource chronic pollution and microbial community composition and potential function were investigated using different chemical parameters (e.g., Al, Cu and Pb levels) and Illumina sequencing.

Results: We found that the sediments were more contaminated than the water, as expected. However, water samples showed less microbial species diversity than sediments and both habitats displayed different microbial communities. Within each of these habitats, pollution had no effect on alpha-diversity but shaped the composition (beta-diversity) of the microbial communities. In contrast to the taxonomic beta-diversity patterns, pollution did not influence the overall functional profiles of the microbial communities, which suggests a decoupling between taxonomy and function. However, certain functional groups were affected by chronic pollution. For instance, photoautotrophic taxa (in water) and taxa involved in the metabolism of nitrogen (in sediments) were under-represented in contaminated samples. These results add to the body of knowledge suggesting that chronic pollution affects microbial community composition and microbial services, such as primary productivity, in aquatic environments.

PW046 Selecting for antibiotic production in micro-droplets

Einat Tamar[1], Roy Kishony[1;2]

[1]*Technion - Israel Institute of Technology, Biology, Haifa, Israel*
[2]*Technion - Israel Institute of Technology, Computer Science, Haifa, Israel*

Background: A major challenge in laboratory evolution is generating conditions that select for evolution of antibiotic production. Antibiotic production genes are ubiquitous in the natural environment, presumably providing their carriers advantage in competition against neighboring susceptible species. However, in typical laboratory settings of well-mixed environments, the advantage of antibiotic production is limited due to dispersion of the drug away from producer cells which reduces local drug concentration as well as shares its benefit with resistant non-producing cheaters.

Objectives: To develop and evaluate a method to select in favor of antibiotic production by encapsulating producer cells with a target susceptible species in small microenvironments.

Methods: We focus on the lantibiotic subtilin, a pore-forming antimicrobial peptide produced by *B. subtilis,* as a model antibiotic with high potential for chemical diversification. To allow selection for subtilin production, we developed an experimental system in which we compete subtilin producers and target cells in a large set of ~10^9 isolated water-in-oil micro-droplets (~30pL each).

Results: First, we demonstrate selective advantage for production. Co-culturing differently fluorescently labeled subtilin producers and non-producers with susceptible cells in droplets, we find that a single producer introduced into a droplet overtakes the droplet population and gains advantage in growth compared to a similar non-producer cell. These results open the door for evolution of producers of modified subtilin capable of overcoming subtilin resistant cells, through successive growth cycles of producers with resistant cells in droplets.

PW047 Evidence of wheat plant ability to control Pseudomonas secondary metabolism

Laura Rieusset[1], Marjolaine Rey[1], Florence Gerin[2], Daniel Muller[2], Claire Prigent-Combaret[2], Gilles Comte[1]

[1]Université Claude Bernard - Lyon 1, UMR CNRS 5557 - Laboratoire d'Ecologie Microbienne - CESN, Villeurbanne, France
[2]Université Claude Bernard - Lyon 1, UMR CNRS 5557 - Laboratoire d'Ecologie Microbienne, Villeurbanne, France

Background: The plant root adhering soil houses an important microbial community. Roots exude a wide variety of secondary metabolites able to attract and/or control a large diversity of microbial species. In return, among the root microbiota, some bacteria are able to promote plant development, using plant growth-promotion and plant protection properties. These bacteria are named PGPR for Plant Growth-Promoting Rhizobacteria. Some PGPR belong to the *Pseudomonas* genus. *Pseudomonas* are known to produce a wide diversity of secondary metabolites that could have biological activity on the host plant and other soil microorganisms. But yet, the impact of host plant on *Pseudomonas* secondary metabolism is still poorly understood.

Objectives: The aim of our project is to better understand the impact of the host plant on secondary metabolite production by fluorescent *Pseudomonas* strains.

Methods: A metabolomic approach was developed in order to decipher how plant may modulate secondary metabolites production in *Pseudomonas*. Five different fluorescent *Pseudomonas* strains were thus cultivated in the presence of root extracts of three wheat genotypes, at low concentration. This experimentation allows us to evaluate the impact of root metabolites on *Pseudomona*s secondary metabolism.

Results: Analysis of our metabolomic workflow revealed that the production of several *Pseudomonas* secondary metabolites were significantly up- or down-regulated when bacteria were cultivated with root extracts. This shows that wheat root metabolites may act as signal compounds and modulate *Pseudomonas* secondary metabolism, including metabolites involved in plant stimulation and plant protection properties. Interestingly, root extract modulation differs according to wheat genotypes and *Pseudomonas* strains.

PW048 Modelling water quality through faecal microbial indicators in a Mediterranean stream

Miriam Pascual-Benito[1], Elisenda Ballesté[1], Daniel Nadal-Sala[2], Cristina García-Aljaro[1], Anicet R. Blanch[1], Francisco Lucena[1]

[1]*University of Barcelona, Genetics, Microbiology and Statistics, Barcelona, Spain*
[2]*University of Barcelona, Evolutionary Biology, Ecology and Environmental Sciences, Barcelona, Spain*

Background: Water scarcity in the Mediterranean area evidences the need for the development of tools to evaluate and improve water management strategies.

Objectives: The aims of this research were to study the seasonal dynamics of five faecal microbial indicators (FMI) in a Mediterranean stream affected by a wastewater treatment plant (WWTP); to assess the assimilation distance (AD) of WWTP impacts and to model self-depuration dynamics.

Methods: FMI were assessed in a Mediterranean stream through different seasonal periods. A statistical correlative model was developed between their decay rates and two main environmental drivers (temperature and river flow after WWTP), and it was later used to evaluate the AD.

Results: A consistent increase in the concentration of all indicators after WWTP effluent and seasonal differences downstream of the WWTP were detected. During the dry period (summer) the observed decay rates were at their highest, while during the wet period (spring, autumn and winter) were at their lowest. This seasonal trend indicates the river's higher capacity to self-depurate during the summer. The highest modelled AD was found for somatic coliphages (~7 km) during spring months, while the lowest ones were found for E. coli and GA17 bacteriophages during summer months (~0.5 km). The AD model may be applied in a larger number of Mediterranean drainage basins to evaluate the WWTP impacts and may allow for their description and comparison for any given indicator in relation to changes in environmental drivers. It could be especially useful to evaluate the effect of climate change.

PW049 Quorum sensing affects prophage induction and biofilm formation in V. anguillarum

Mads Frederik Hansen[1], Demeng Tan[2], Henriette Lyng Røder[1], Mette Burmølle[1], Mathias Middelboe[3], Sine Lo Svenningsen[2]

[1]*University of Copenhagen, Department of Biology, Section of Microbiology, Copenhagen, Denmark*
[2]*University of Copenhagen, Department of Biology, Section for Biomolecular Sciences, Copenhagen, Denmark*
[3]*University of Copenhagen, Department of Biology, Marine Biological Section, Helsingør, Denmark*

Background: Outbreaks of Vibriosis by fish pathogenic *V. anguillarum* can have immense economic consequences in the aqua culture industry. Biofilm formation contribute to the adhesion of *V. anguillarum* to the surface of fish and also makes infections harder to eradicate due to the emergent properties of biofilm i.e. increased tolerance towards antimicrobials. Quorum sensing (QS) is known to orchestrate bacterial behaviour including biofilm formation, and within *Vibrios* there are examples of both QS-activated and -repressed biofilm formation. Additionally, bacteriophages integrated into their hosts chromosome, known as prophages, can affect the bacterial phenotype. Here, we present how QS and H20-like prophages affects biofilm formation in *V. anguillarum.*

Objectives: To analyse how QS and H20-like prophages affect biofilm formation and composition in *V. anguillarum.*

To establish if prophage induction and QS is linked in *V. anguillarum*

Methods: By in-frame deletion of genes encoding the QS master regulator (Δ*vanT*) and response regulator (Δ*vanO*) in *V. anguillarum*, we have constructed otherwise isogenic density-independent mutant strains locked in states of low- and high-cell densities, respectively.

Confocal laser scanning microscopy and staining methods are used to examine biofilm formation of the different mutants.

Results: The biofilm phenotypes of these mutants show that QS strongly represses biofilm formation in *V. anguillarum*. Remarkably, we find that the amount of free H20-like phages found in the supernatant is also QS regulated. Prophage-free strains harbouring the QS-mutations mentioned above indicate that the prophage is stimulating biofilm formation at low cell densities.

PW050 Phenotypic and molecular characterisation of Staphylococcus aureus associated with bovine subclinical mastitis and teat skin colonization

Carina Matias[1], Yasser S. Mahmmod[2], Line Svennesen[3], Hanne Ingmer[3], Morten Sommer[1]

[1]Technical University of Denmark - Novo Nordisk Foundation Center for Biosustainability, Denmark
[2]IRTA, Centre de Recerca en Sanitat Animal (CReSA), Campus de la Universitat Autònoma de Barcelona, Spain
[3]Department of Veterinary and Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark

Background: *Staphylococcus aureus* is a versatile pathogen accounting for a wide spectrum of infections in humans and causing intramammary infections in dairy cows. Bovine mastitis is the main reason for antibiotic use in dairy farms, which can contribute to the rise of antibiotic resistance. The presence of *S.aureus* in dairy farms is also a potential risk, since it can be transmitted from animals to humans.

Objectives: The aim was to determine the phenotypic and molecular characteristics of *S.aureus* associated with bovine subclinical mastitis and teat skin colonization within a dairy herd context.

Methods: The susceptibility patterns of 59 *S. aureus* isolated from both cow's teat skin (n=30) and aseptically collected milk (n=29) in a panel of 13 relevant antibiotics, was determined. Genomic data was obtained recurring to Illumina HiSeq 2500 and the multilocus sequence type (MLST), *spa* type and genes related with antibiotic resistance characterized.

Results: A high prevalence of antibiotic resistance was observed. Resistance rates were: Penicillin (97%), Cloxacillin (95%), Sulfonamide/Trimethoprim (95%), Amoxicillin (95%), Erythromycin (95%), Tetracycline (44%), Fusidic Acid (98%), Oxacillin (92%), Clindamycin (100%), Vancomycin (93%) and Linezolid (98%), Norfloxacin (2%) and Enrofloxacin (27%). Seven different MLSTs were identified, with ST151 being the most prevalent (47%), followed by ST3891 (7%), ST504 (4%), ST133 (3%), ST1380 (2%), ST479 (1%) and ST3897 (1%).
Our isolates showed a high level of antimicrobial resistance. Although the MLST lineages observed are considered cattle specific, these multi-resistant isolates represent a reservoir of antibiotic resistance genes that could contribute to the dissemination of antibiotic resistance.

PW051 Isolation and high-throughput screening of xenobiotic degrading bacterial and fungal strains

Giulia Spini[1], Federica Spina[2;2], Anna Poli[2], Ilaria Re[3], Carla Gramellini[4], Cristina Menta[5], Giovanna Cristina Varese[2], Edoardo Puglisi[1]

[1]Università Cattolica del Sacro Cuore, Department for sustainable food processes (DiSTAS), piacenza, Italy
[2]Mycotheca Universitatis Taurinensis- Università di Torino, Department of Life Sciences and Systems Biology, torino, Italy
[3]Consorzio Italbiotec, Milano, Italy
[4]Arpae Emilia Romagna, Bologna, Italy
[5]Università di Parma, Department of Chemistry, Life Sciences and Environmental Sustainability, Parma, Italy

Background: Autochthonous bioaugmentation, by exploiting the indigenous microorganisms of the contaminated environment to be treated, can represent a successful bioremediation strategy.

Objectives: In this perspective, hydrocarbons degrading fungi and bacteria were isolated from a soil strongly polluted by mixtures of aliphatic and polycyclic hydrocarbons and screened by cultural and molecular methods.

Methods: Three consecutive enrichments were carried out on different target pollutants using soil samples from different soil depths, and analysed by high-throughput sequencing (HTS) of microbial biomarkers.

Results: A total of 95 bacterial and 94 fungal strains were isolated after the selective procedure, mainly ascribed to *Pseudomonas* genus followed by *Sphingobacterium*, *Bacillus*, *Stenothrophomonas*, *Achromobacter* and *Serratia* for bacteria. As for fungi, *Fusarium* was the dominant genus followed by *Trichoderma* and *Aspergillus*. Molecular analyses corroborated the culture-dependent analyses in terms of most abundant microbial species. The results highlighted that for both bacteria and fungi the pollutant had a partial shaping effect on the composition of enriched communities. All the isolated fungi and bacteria were further analysed by an innovative miniaturized approach in 96 multiwell plates. The growth rate and/or metabolic activity were evaluated in the presence of target contaminants as sole carbon source to identify the strains with best adaptation and degradation skills. Several bacterial and fungal strains showed remarkable degradative capabilities towards pollutants displaying higher or equal growth respect to control (glucose). These isolates adapted their metabolism to exploit also different aliphatic and aromatic compounds, even more efficiently than glucose, and are now being exploited for environmental bioremediation.

PW052 Characterization of two sphingobium sp. strains that degrade cadusafos, an organophosphorus insecticide

Jae-hyung Ahn[1], in cheol park[1], Jaehong you[1], byeong-hak han[1]

[1]national institute of agricultural sciences, rural development administration (rda), agricultural microbiology division, wanju-gun, jeollabuk-do, Korea, Rep. of South

Background: Although cadusafos, an aliphatic organophosphorus (OP) insecticide, is not approved by the European commission, it is used in several countries and sometimes detected as a residue in soils and agricultural products.

Objectives: In this study, two cadusafos-degrading *Sphingobium* sp. strains, Cam5-1 and K22212, were isolated and characterized for use as detoxifying agents of the insecticide.

Methods: The cadusafos-degrading bacterial strains were isolated from agricultural soils using an enrichment method.

Results: Both strains degraded 100 mg L^{-1} of cadusafos in mineral medium within 12 h through a common metabolite, which was supposed to be dimerized thiophosphates based on its molecular weight. Degradation of cadusafos caused cell growth for Cam5-1 but not for K22212. Cam5-1 and K22212 degraded cadusafos in soil (15 mg kg^{-1} dry soil) within 2 and 5 days, respectively. Both strains also degraded ethoprophos, malathion, profenofos, fosthiazate, phenthoate and phorate but not chlorpyrifos and diazinon, indicating that they are specialized for degradation of OP insecticides with at least one single bond connecting phosphorus and sulfur atoms (P-S bond). The degradation rates of the two strains were the largest for ethoprophos, followed by cadusafos, fosthiazate, malathion, profenfos, phenthoate, and phorate. These results indicate that the bacterial strains are effective degraders of OP insecticides with P-S bonds, and in particular, Cam5-1 is promising for removal of the OP insecticides in soils. The sequencing of the genome of Cam5-1 resulted in five circular contigs, which have the total length of 4,371,875 bp, 4,240 protein-coding genes, 53 tRNAs and 6 rRNAs.

PW053 Single-cell real time analysis of the anaerobic bacterium Desulfovibrio vulgaris demonstrates population heterogeneity that decreases with increasing pH stress

Ciara Keating[1], Yanqing Song[1], Ayo Ogundero[1], Rebeca Gonzalez-Cabaleiro[1], Huabing Yin[1], William Sloan[1]

[1]*University of Glasgow, School of Engineering, Glasgow, United Kingdom*

Background: Anaerobic digestion (AD) is a multi-step process carried out by a consortium of anaerobic microorganisms. Our understanding is built upon population-based studies that measure the cumulative activity and function of the cells within. Yet it is becoming apparent that variability in phenotypes can occur even within single species. Considering the biodiversity of individual cells of vital species and how these can affect the AD process is fundamental to understanding these systems and implementing into engineering practice.

Objectives: *Desulfovibrio vulgaris* (*D.v.*) cells were monitored during growth in optimum conditions and under pH stress. The heterogeneities in individual cells and the distribution of growth rates was assessed across the conditions and compared to the Gibbs Free Energy potential (ΔG^o) of available energy for the reactions.

Methods: *D.v.* was cultured in a microfluidic device culturing system under continuous flow of medium on a microscope platform in an anaerobic incubator maintained at 37°C. Using time-lapse imaging the growth rate of individual cells were monitored in optimal conditions (pH 7; ΔG^o -57.84) and less favourable conditions (pH 6.5; ΔG^o -56.75). Cells that had grown and were not near neighbouring cells were considered for analysis.

Results: The actively growing population in each condition consisted of <20% of the total number of cells. At pH 7 cells had a lag time of 22 hr and growth rates varied from 0.01 – 0.2 μ hr^{-1}. At pH 6.5 cells had a longer lag time (100 hr) but the growth rates were more homogenous (~ 0.2 – 0.3 μ hr^{-1}).

PW054 metabarcoding analysis of environmental samples collected during cocoa beans fermentation

Lucie Farrera[1], benjamin guibert[1], florentin constancias[1], yasmine hamdouche[2], mirna suarez quiroz[3], didier montet[1], Corinne Teyssier[4]

[1]cirad, umr qualisud, montpellier, France
[2]laboratory of biology and microbial systems, normal superior school, algiers, Algeria
[3]instituto tecnologico de veracruz, veracruz, Mexico
[4]university montpellier, umr qualisud, montpellier, France

Background: Cocoa beans fermentation is the first step in the chocolate production. This post-harvest process lasts usually between 5 to 7 days and different microorganisms including yeasts, Lactic Acid Bacteria, Acetic Acid Bacteria are successively implied. Currently their roles are well-documented. Since few years, metabarcoding approaches performed on microbial DNA collected on cocoa beans at different times of fermentation showed the presence of many others bacteria (for example *Enterobacteriaceae*). Few data are available on their origin and their potential roles in the fermentation process.

Objectives: The aim of this study was to determine the origin of these bacteria by comparing the microbial ecology from environmental (closed to the cocoa beans) and cocoa beans samples collecting during a same fermentation process.

Methods: About one hundred environmental (originating from pods, machetes, fermentation boxes, farmers hands) and cocoa beans samples were collected in November 2018 in Mexico from spontaneous fermentation process. The environmental sampling was carried out in sterile conditions by surface swabbing. Microbial DNA was extracted and then metabarcoding analysis on 16S rDNA was performed.

Results: This preliminary metabarcoding approach showed different origins according to the bacterial genus. Others sampling should be performed in order to confirm these first results. The main perspective of this study is to precise the role of these microorganims during cocoa beans fermentation

PW055 Phylogenetic analyses of antibiotic-producing Streptomyces sp. isolates obtained from the stingless-bee Tetragonisca angustula

Juan Carlos Cambronero-Heinrichs[1], Bernal Matarrita-Carranza[2], Catalina Murillo-Cruz[2], Emanuel Araya-Valverde[3], Max Chavarria[3], Adrian Pinto-Tomás[2]

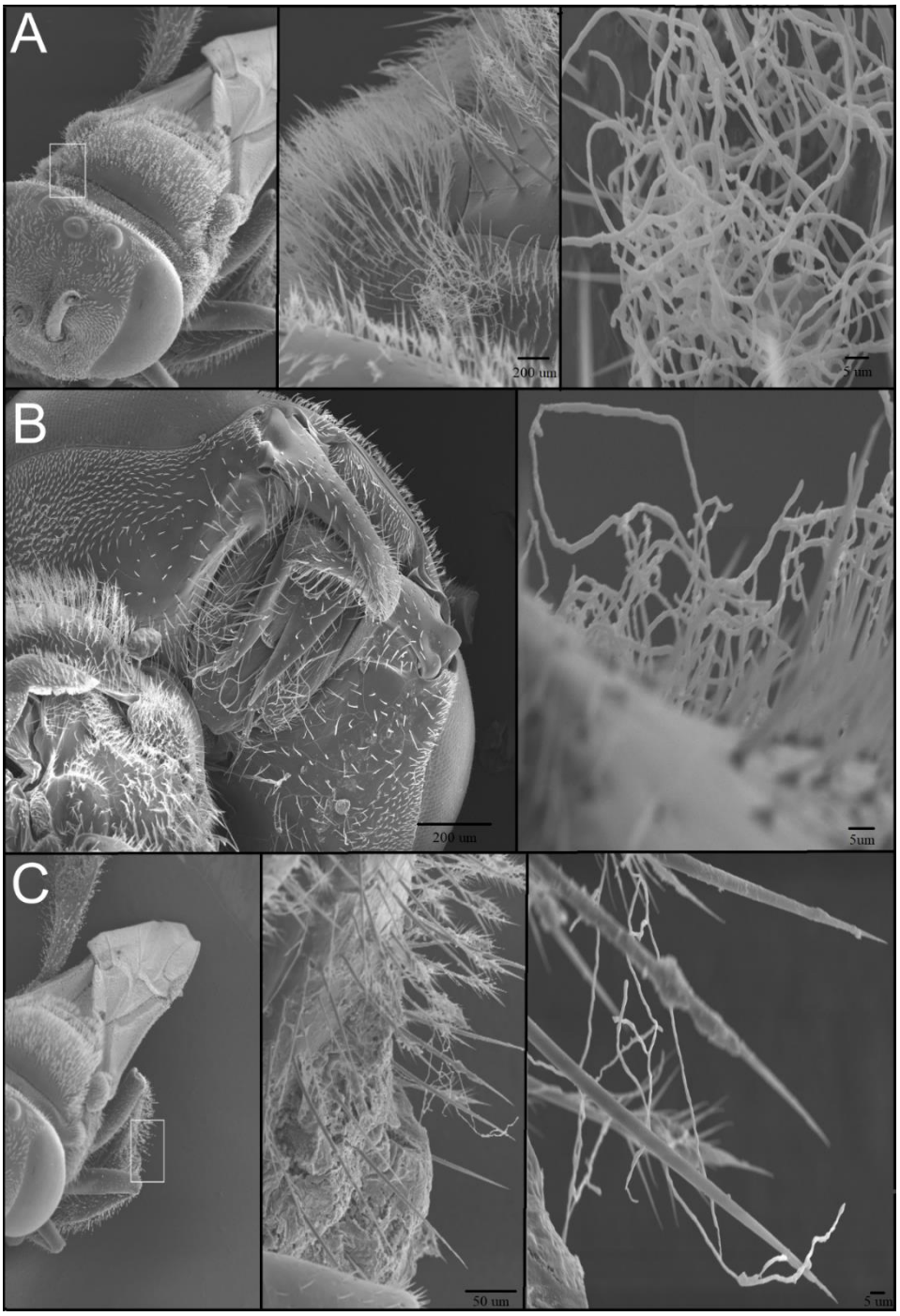
[1]*Universidad de Costa Rica, Centro de Investigación en Contaminación Ambiental, San Jose, Costa Rica*
[2]*Universidad de Costa Rica, Centro de Investigación en Estructuras Microscópicas, San Jose, Costa Rica*
[3]*Centro Nacional de Innovaciones Biotecnológicas, CENIBiot, Costa Rica*

Background: Many insects are associated with actinobacteria in protective symbiosis where antimicrobial metabolites inhibit host pathogens. However, the microbiota of neotropical insects is poorly explored. *T. angustula* is a meliponid-bee widely distributed in Latin America, its honey is exploited because of its ethno-pharmacological properties, and its antimicrobial activity has been demonstrated. Even though *Streptomyces* spp. have been cultured from stingless-bees, little is known about their role in Meliponini.

Objectives: We examined the association between culturable actinobacteria and *T. angustula,* and evaluated the isolates' antimicrobial potential.

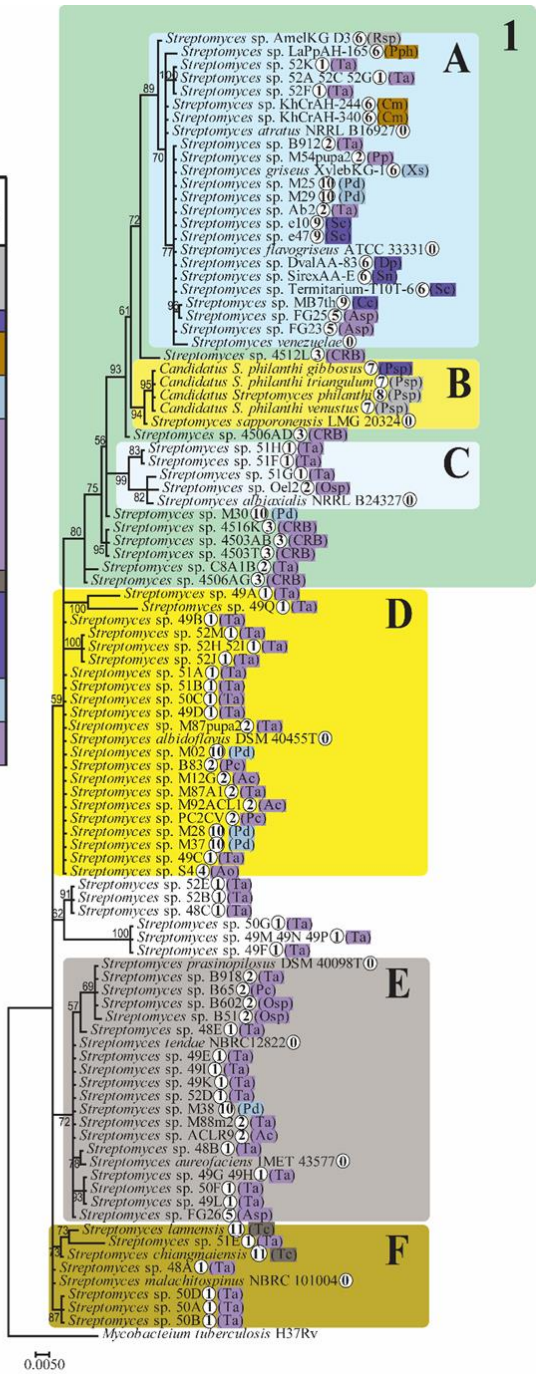
Methods: We isolated 51 actinobacteria from adult bees and the hive of *T. agustula*. Then, we performed a 16S rRNA phylogenetic analysis that clusters the bacteria to lineages of host-associated *Streptomyces.* Also, the isolates were classified according to their antibacterial activity against human pathogens, measured by a growth inhibition test based on agar diffusion. Finally, SEM analysis was performed on adult bees and hive substrates to evaluate bacterial presence.

Results: More than 50% of our isolates exhibit antimicrobial activity, mainly to Gram-positives and fungi and only two against Gram-negatives. Additionally, electron micrographs of bees revealed patches of *Streptomyces*-like filaments loosely attached on their surface. That suggest that *T. angustula* possibly uptake/transfer actinobacteria from the environment. Our results provide new insights regarding the microbiota associated to *T. angustula* and justify future studies exploring the microbial community associated to the hive and the possible exchange of microbes with the crops they pollinate.


A
200 um
5 um
B
200 um
5um
C
50 um
5 um

Geographic Distribution	Insect	Abbreviation ()
Europe	*Reticulitermes* sp.	Rsp
	Philanthus triangulum triangulum	Psp.
	Philanthus venustus	Psp.
North America	*Philanthus gibbosus*	Psp.
Africa	*Crematogaster margaritae*	Cm
	Petalomyrmex phylax	Pph
Worldwide, Invasive.	*Polistes dominulus*	Pd
Central and South America	*Agelaia cajennensis*	Ac
	Polybia plebeja	Pp
	Acromyrmex octospinosus	Ao
	Paraponera clavata	Pc
	Odontomachus sp.	Osp
	Allomerus sp.	Asp
	Tetragonisca angustula	Ta
Asia	*Tetragonilla collina*	Tc
North America	*Sirex noctilio*	Sn
	Chalybion californicum	Cc
	Sceliphron caementarium	Sc
	Dendroctonus ponderosae	Dp
Worldwide, Invasive	*Xyleborinus saxeseni*	Xs
Central and South America	Costa Rican Beetles	CRB

Origin of the strains sequences	Symbol, **Bold Numbers**
Free living Actinobacteria	⓪
This Study	①
Matarrita-Carranza et al. 2017	②
Vargas-Asencio et al. 2014	③
Seipke et al. 2011	④
Seipke et al. 2012	⑤
Book et al. 2016	⑥
Kalthenpoth et al. 2006	⑦
Kalthenpoth et al. 2012	⑧
Poulsen et al. 2011	⑨
Madden et al. 2013	⑩
Promnuan et al. 2013	⑪



PW056 Growth phase dependent nematocidal activity in Bacillus turingiensis strains from natural samples

Iva Atanaskovic[1;2], Jelena Lozo[3], Slavisa Stankovic[1], Djordje Fira[1]

[1]*Studentski trg 16, Faculty of Biology, University of Belgrade, Belgrade, Serbia*
[2]*New Biochemistry Building, Department of Biochemistry, Oxford, United Kingdom*
[3]*Faculty of Biology, University of Belgrade, Belgrade, Serbia*

Background: Competition between domestic plants and parasitic nematodes is one of the biggest challenges in agrobiotechnology. Currently available nematocidal products are toxins with a broad activity range, but that are soluble in water and very harmful for the environment. Thus there is a need to develop new, environmental-friendly nematocides, such as those of microbial origin. *Bacillus thuringiensis* (Bt) is a bacterium that can synthesise different parasporal crystal proteins (Cry) in a growth-phase dependent manner, where some Cry proteins can act as potent nematocides.

Objectives: To identify novel Bt strains with nematocidal activity; to investigate *cry* gene composition and growth-phase dependence of nematocidal activity in these strains.

Methods: In this study, we identified Bt strains from a collection of natural *Bacillus* spp. isolates using a novel polyphasic approach, that includes sequence analysis of 16S rRNA and *tuf* genes, detection of several *cry* genes and detection of nematocidal activity against *Caenorhabditis elegans*. We further investigated how does the growth phase in which bacterial cells are harvested influence the rate of nematocidal activity via a *C. elegans* killing assay.

Results: Our findings show that different Bt strains, that contain distinct combinations of *cry* genes, split into two groups of growth phase dependent nematocidal activity profiles. We further show that combining two strains from these two groups enables complete abolishment of nematodes in a *C. elegans* killing assay. These findings can give new perspectives on the application of Bt strains in biocontrol of parasitic nematodes.

PW057 Plant-microbe association in Taxus wallichiana Zucc. (Himalayan Yew)

Priyanka Adhikari[1], Anita Pandey[1]

[1]*GBPNIHESD Uttarakhand, Environmental microbiology, Almora, India*

Background: Himalayan yew (*Taxus wallichiana* Zucc.; Family Taxaceae), due to its anticancer property, is a highly valued medicinal conifer of Indian Himalaya. Mentioned in IUCN red list, it is a biodiversity conservation priority species. Insight on root microbiome will help in understanding the contribution of plant-microbe association in growth and protection of the host under low temperature environment.

Objectives: Investigation on the endophytic microorganisms associated with root microbiome of *T. wallichiana* with particular reference to their functional aspects.

Methods: Root colonization following surface sterilization and microscopy, **i**solation and identification following polyphasic approach, and characterization for plant growth and biocontrol properties following standard procedures.

Results: Microscopic observations revealed colonization of *T. wallichiana* roots by endophytic bacteria and fungi. Prolonged dip treatment in distilled water helped in removal of melanin like substances from the roots and resulted in clarity with respect to structures (size and shape) of endophytes. Colonization of fungal mycelium was recorded up to 84 %, followed by arbuscular mycorrhizae (52 %) and dark septate endophytes (34 %). The culturable bacterial species belonged to *Bacillus* and *Burkholderia* while the fungal species belonged to *Aspergillus* and *Penicillium.* These endophytes showed ecological resilience through their tolerance to wide temperature, pH, and salt concentration. Functionally, these endophytes possessed plant growth and biocontrol properties such as phosphate solubilization and antagonism. Such detailed studies will have implications in understanding the role of endosymbiosis in plant growth growing under low temperature environments.

PW058 Diversity and metabolism of xylose and glucose fermenting microbial communities in sequencing batch or continuous culturing

Julius L Rombouts[1], Galvin Mos[1], David G Weissbrodt[1], Robbert Kleerebezem[1], Mark C M Van Loosdrecht[1]

[1]*Delft University of Technology (TU Delft), Delft, Netherlands*

Background: Enrichment culturing can elucidate mechanisms driving microbial selection and diversity. Here, selection and stoichiometries were studied during mixed-culture fermentation of glucose and xylose, the two most abundant carbohydrates in agro-waste streams. Sequencing batch reactors (SBRs) and continuous-flow stirred tank reactors (CSTRs) fed with solely xylose or glucose where used, assessing this impact. One substrate is limited and competed for, thus one microorganism was expected to dominate per enrichment.

Objectives:

1. Compare the stoichiometry and kinetics for glucose and xylose fermentation
2. Analyze the community structure to evaluate the diversity hypothesis

Methods: The reactors were operated anaerobically at 30±0.5°C and pH 8.0±0.1, with a hydraulic retention time of 8 h, inoculated with bovine rumen. We used a mineral medium supplemented with either glucose or xylose at 4 g/L. Main products were quantified by HPLC, GC, and photometric off-gas analysis. Stoichiometries were verified with balancing. Community compositions were analyzed by 16S rRNA gene amplicon sequencing, full 16S clone libraries, and 16S rRNA-targeted FISH.

Results:

1. Acetate/ethanol formation was observed in the SBRs and the xylose CSTR, linked with a high biomass specific substrate uptake rate (q_s^{max})
2. The SBR enrichments showed a 2-3 times higher q_s^{max} and 50% lower biomass yield on ATP than the CSTR enrichments
3. In the SBRs *Citrobacter freundii* (xylose) and *Enterobacter cloacae* (glucose) dominated at >80%
4. In the CSTRs *Clostridium intestinale* dominated for glucose, while surprisingly for xylose three populations coexisted

Overall, more diversity was observed than expected, indicating non-competitive mechanisms shape microbial communities.

PW059 Dominant coral bacterium Endozoicomonas acroporae metabolizes DMSP

Kshitij Tandon[1], Pei-Wen Chiang[1], Wen-Ming Chen[2], Sen-Lin Tang[1]

[1]*Biodiversity Research Center, Academia Sinica, Taipei, Taiwan*
[2]*Laboratory of Microbiology, Department of Seafood Science, National Kaohsiung Marine University, Kaohsiung, Taiwan*

Background: Reef-building corals such as those in genus *Acropora* are one of the major producers of dimethylsulfoniopropionate (DMSP), and dominant coral-associated bacteria *Endozoicomonas* species have been hypothesized to play active roles in the global sulfur cycle, effectively metabolizing DMSP to dimethylsulfide (DMS). However, no sequenced *Endozoicomonas* genomes to date harbor genes for DMSP metabolism.

Objectives: i) To assemble and annotate genomes of two strains (Acr-1 and Acr-5) of *Endozoicomonas acroporae*. ii) To identify and functionally characterize the DMSP metabolism gene(s). iii) To perform comparative genomic analysis of genus *Endozoicomonas.*

Methods: High-quality draft genomes of *E. acroporae* strains (Acr-1 and Acr-5) were assembled by CLC-genomics workbench and annotated with Rapid Annotation using Subsystem Technology (RAST). We used RT-qPCR and Gas Chromatography-Mass Spectrometry (GC-MS) to quantify *dddD* gene expression and the amount of DMS released when cultures are grown in a DMSP rich-environment. Pan-genome analysis was performed using Roary.

Results: We assembled high-quality draft genomes of *E. acroporae* strains (Acr-1 and Acr-5) and annotated the first DMSP lyase—a *dddD* gene homolog capable of metabolizing DMSP to DMS in all strains of *E. acroporae.* RT-qPCR based expression analysis of the *dddD* gene and GC-MS based quantification of DMS release confirmed *E. acroporae* species' ability to metabolize DMSP and, thus, their role in the global sulfur cycle. Further, high genomic divergence within the genus and its adaptation to marine environments was confirmed by identifying the reduced core-genome and high proportion of oxidative stress responsive genes in all genome of genus *Endozoicomonas* respectively.

PW060 Whole Genome Sequencing analysis of Multi Drug Resistant Shewanellaceae and Vibrionaceae isolates: a One-Health survey in Northeastern Italy

Vanessa Zago[1], Giovanni Malerba[2], Cristina Patuzzo[2], Patrice Nordmann[3], Maria Del Mar Lleò Fernandez[1], Laurent Poirel[3]

[1]University of Verona, Department of Diagnostic and Public Health - Microbiology Unit, Verona, Italy
[2]University of Verona, Department of Neuroscience, Biomedicine and Movement Science, Verona, Italy
[3]University of Fribourg, Medical and Molecular Microbiology Unit, Faculty of Science and Medicine, Fribourg, Switzerland

Background: Antimicrobial resistance is a global concern in Multi Drug Resistant Gram negative bacteria infections not only in health-care settings but also in environmental and veterinary ones.

Objectives: In a One-Health perspective we studied: (1) the resistome and mobilome of 12 MDR *Shewanellaceae* and *Vibrionaceae* strains; (2) the protein characterization of two beta-lactamases; (3) the phylogenetic analysis of predicted class C and D beta-lactamases protein sequences**.**

Methods: Twelve marine strains were selected from a 41 MDR collection for *de novo* sequencing using Illumina NextSeq® 500 platform. Resistome, mobilome and phylogenetic analysis were performed. Cloning of $bla_{\text{CARB-23}}$ and bla_{ampC} genes from *Vibrio parahaemolyticus* and *V. anguillarum* strains was carried out.

Results: Resistance towards β-lactams ($bla_{OXA\text{-}55\text{-}like}$, bla_{ampC}, $bla_{CARB\text{-}23}$ and *mexAB-OprM*); quinolones (*qnrA7*, *qnrA3*, *qnrVC6*); colistin (*eptA*) and chloramphenicol (*cat*) was observed. Several efflux pumps (*mepAB-OprM*, *mdtK*, *emrAB-TolC*) confer resistance to numerous drugs. Insertion Sequences family transposases such as IS*630*, IS*110*, IS*3*, IS*200*/IS*605,* IS*5*/IS*1182* were detected. IS*As1,* IS*Spu13* and IS*Spu14* were found in *Vibrio* spp. genomes but IS*Spu8* and IS*Spu18* only in *Shewanella* spp. ones. The phylogenetic analysis of ampC protein sequences showed the belonging to *Shewanella algae* and *V. anguillarum* groups. A Neighbor-joining phylogenetic tree for 58 OXA-like class D β-lactamases was created. Sequences were compared between the clinical ones present in *Acinetobacter* spp. and the environmental ones present in *Shewanella* spp. OXA-55-like protein sequences produce a cluster within the *Shewanella* OXA groups. Purification of CARB-23 and ampC is ongoing. In conclusion, marine strains maintain ARGs reservoir in the environment.

PW061 Bacterial community composition and diversity indicate health status of the aquaculture environment in european sea bass (dicentrarchus labrax) farms

Anamarija Kolda[1], Maja Mucko[2], Zrinka Ljubešić[2], Lorena Perić[1], Irena Vardić Smrzlić[1], Jakov žunić[1], Damir Kapetanovic[1]

[1]Ruđer Bošković Institute, Department for Marine and Environmental Research, Zagreb, Croatia
[2]Faculty of Science, University of Zagreb, Department of Biology, Zagreb, Croatia

Background: Aquaculture is a rapidly growing industry accounting for 44% of total fish production worldwide. Nonetheless, yields are constricted by high mortality rates due to diseases. Bacterial community in the water is essential for water purification, water quality and fish health. In sediment, it has important role in organic matter decomposition and nutrient cycling. Insights gained from these communities can be used as a tool in assessing health and management of the aquaculture environment.

Objectives: Objective of the present study was to describe the water and sediment bacterial community on the farms and their respective control sites, link them to the environmental characteristics and detect season and site dependent patterns in bacterial composition and diversity.

Methods: Sampling was conducted in spring, summer, autumn and winter in 2017 on 2 commercial European sea bass (*Dicentrarchus labrax*) farms and control sites: in Central Eastern Adriatic Sea and in South Eastern Adriatic Sea. Physico-chemical parameters were measured *in situ*. Total DNA was extracted from water and sediment samples and 16S rRNA high-throughput sequencing was performed, followed by bioinformatics and statistical analysis.

Results: On both farm sites in water column, core community on phyla level was identified as *Proteobacteria*/*Cyanobacteria*/*Bacteroidetes*. Sediment core community for both farm sites was *Proteobacteria*/*Plantomycetes*/*Bacteroidetes*/*Actionbacteria*. Control sites corresponded to farm sites regarding phyla composition. Interaction between environmental factors and bacterial communities in water and sediment was confirmed statistically. This research adequately responds to the current lack of information on composition and diversity of microbial community in aquaculture systems.

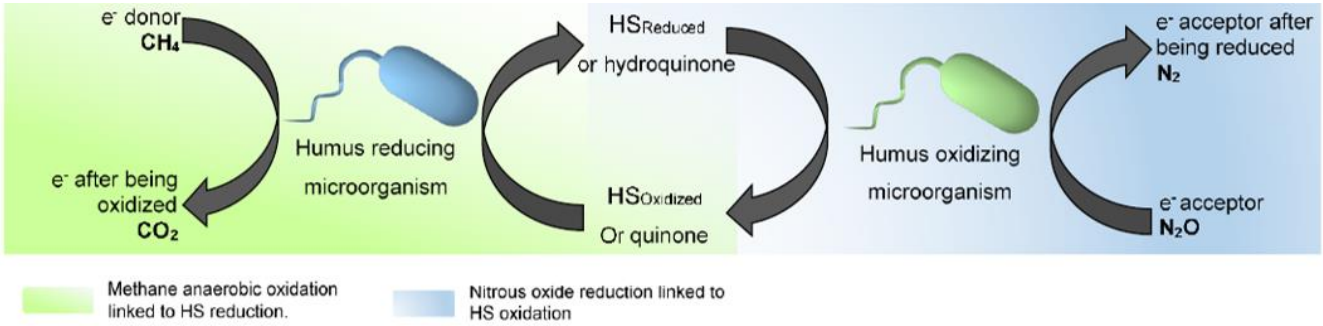
PW062 Humic substances mediate anaerobic methane oxidation linked to nitrous oxide reduction in wetland sediments

Edgardo Iván Valenzuela Reyes[1], Claudia Padilla Loma[1], Nicolás Gómez Hernández[2], Sergio Casas Flores[2], Francisco J Cervantes[1]

[1]*Instituto Potosino de Investigación Científica y Tecnológica A.C., Environmental Sciences, San Luis Potosí, Mexico*
[2]*Instituto Potosino de Investigación Científica y Tecnológica A.C., Molecular Biology, San Luis Potosí, Mexico*

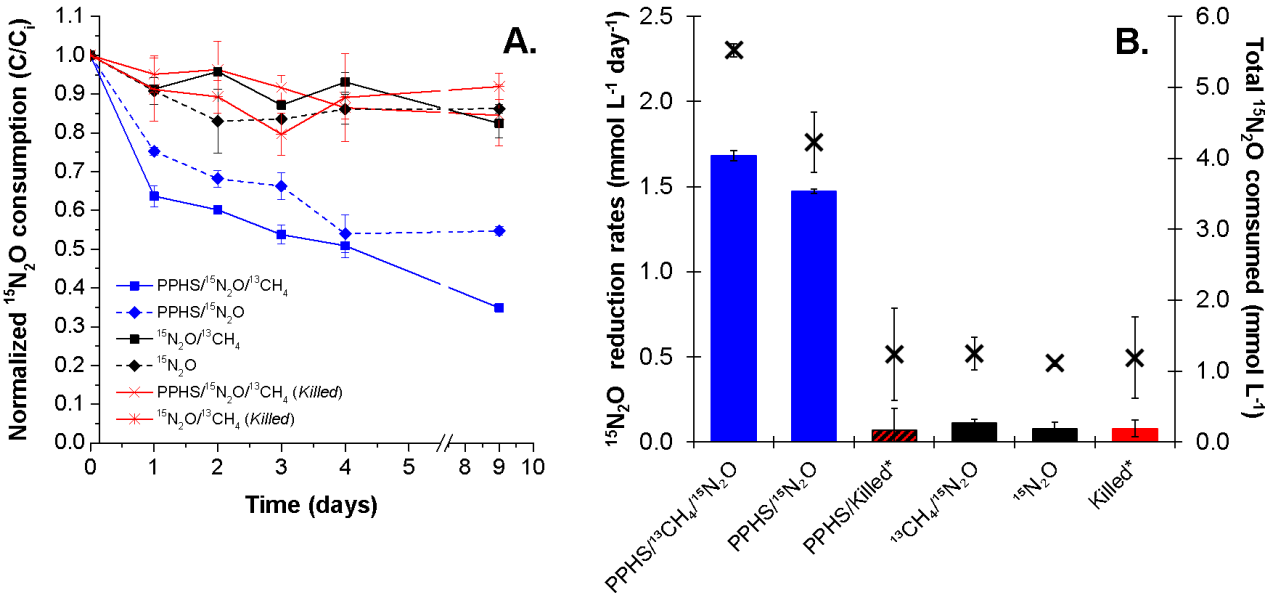
Background: Humic substances (HS) can accept or donate electrons from/to microorganisms since they are rich in redox-active functional moieties. Electrons derived from the oxidation of substrates (such as CH_4) by humus-reducing microorganisms could then be used by humus-oxidizing microorganisms to reduce oxidized compounds (such as N_2O). So far, it is unknown if HS could link the simultaneous consumption of greenhouse gases by shuttling electrons trough different microbial phylotypes.

Objectives: To prove that the anaerobic microbiota of a wetland sediment can perform anaerobic CH_4 oxidation coupled to N_2O reduction via an inter-species electron transfer mediated by HS (Figure 1).



Methods: Microcosms were set-up to track the simultaneous consumption of $^{13}CH_4$ and $^{15}N_2O$ in the presence of HS. Cloning of the gene *nosZ* (N_2O reductase encoding gene) and ILLUMINA sequencing of the total microbiome (16s) were performed with DNA extracted at the end of the experiments.

Results: The highest rates of $^{15}N_2O$ reduction were achieved in the presence of $^{13}CH_4$ and PPHS (Figure 2). $^{13}CH_4$ was oxidized to $^{13}CO_2$ only in the presence of $^{15}N_2O$ and PPHS. This evidence strongly suggests that anaerobic oxidation of $^{13}CH_4$ linked to $^{15}N_2O$ reduction occurred due to the electron transport triggered by HS. *Arenibacter algicola* was identified as the main N_2O reducer according to the *nosZ* sequences obtained. Bacterial and archaeal population abundances showed differences in response to the exposition to each greenhouse gas and to HS. We propose the first mechanism of HS mediating the simultaneous consumption of two potent greenhouse gases (CH_4 and N_2O).


A.
Normalized $^{15}N_2O$ consumption (C/C_i)
Time (days)
PPHS/$^{15}N_2O$/$^{13}CH_4$
PPHS/$^{15}N_2O$
$^{15}N_2O$/$^{13}CH_4$
$^{15}N_2O$
PPHS/$^{15}N_2O$/$^{13}CH_4$ (Killed)
$^{15}N_2O$/$^{13}CH_4$ (Killed)
B.
$^{15}N_2O$ reduction rates (mmol L^{-1} day^{-1})
Total $^{15}N_2O$ comsumed (mmol L^{-1})
PPHS/$^{13}CH_4$/$^{15}N_2O$
PPHS/$^{15}N_2O$
PPHS/Killed*
$^{13}CH_4$/$^{15}N_2O$
$^{15}N_2O$
Killed*

PW063 Acinetobacter lwoffii - environmental strain with unusual characteristics

Joanna Klim[1], Tomasz Walter[1], Marcin Jurkowski[1], Małgorzata Słodownik[1], Urszula Zielenkiewicz[1]

[1]*The Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Department of Microbial Biochemistry, Warsaw, Poland*

Background: Bacteria belonging to *Acinetobacter* are commonly known as human pathogens, but they also play a significant role in various ecological niches, including many types of aquatic and terrestrial environments. Here, we present studies concerning environmental isolate ZS207 belonging to *Acinetobacter lwoffii* species, that has developed numerous mechanisms enabling survival in an environment containing a high level of arsenic and other heavy metals compounds.

Objectives: The aim of this work was to evaluate physiological and genomic characteristics (with emphasis on the plasmidome) of the environmental *A. lwoffii* ZS207 strain isolated from a gold and arsenic mine in Złoty Stok (Poland).

Methods: First, we have sequenced and analyzed, using diverse bioinformatics tools, the genomic DNA. The genome of tested *A. lwoffii* isolate includes 1 chromosome of about 3.5 Mb, 8 plasmids (size ranging between 4 to 17 kb) and 1 megaplasmid (~198 kb).

Second, we have evaluated minimal inhibitory concentrations concerning heavy metals and arsenic salts, antibiotic susceptibility of the *A. lwoffii* ZS207, its salinity tolerance and pH growth range.

Third, we have assessed functionality of 5 identified, complete toxin-antitoxin systems by cloning them in highly unstable pABB35 vector and performing standard plasmid stabilization assay.

Results: Analyzes show the presence of multiple TA systems, transposons and prophages and number of genes conditioning resistance to heavy metals, such as zinc, copper, iron, cadmium and arsenic compounds, which is in line with the observations made during the physiological characterization. These results display that *A. lwoffii* ZS207 can be potentially useful in bioremediation.

PW064 Bacterial communities at contaminated coastal sites in the Baltic Sea - potential as indicators of disturbance

Juanjo Rodríguez[1], Christine Gallampois[2], Sari Timonen[1], Agneta Andersson[3], Hanna Sinkko[1], Peter Haglund[2], Åsa Berglund[3], Matyas Ripszam[4], Daniela Figueroa[3], Mats Tysklind[2], Owen Rowe[5], Erik Björn[2]

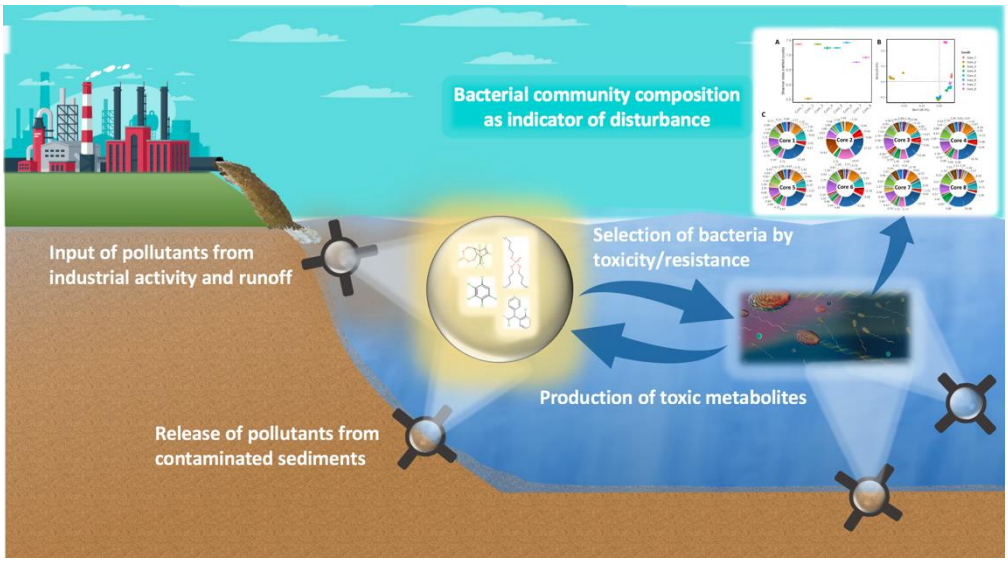
[1]*University of Helsinki, Microbiology, Helsinki, Finland*
[2]*Umeå University, Chemistry, Umeå, Sweden*
[3]*Umeå University, Ecology and Environmental Sciences, Umeå, Sweden*
[4]*MSCi ApS Laboratory, Denmark*
[5]*Helsinki Commission (HELCOM), Helsinki, Finland*

Background: Bacteria inhabit a wide range of environments, and heavily contaminated sediments and waters of the Baltic Sea are no exception. In such environments, complex interactions between the physico-chemical environment and microbial communities exist. The resulting bacterial communities are structured by their environment (e.g. selection by toxicity/resistance) and can in return influence their environment, for example the production of toxic metabolites. While some studies have explored the consumption and metabolism of pollutants by different bacterial taxa or their potential for bioremediation, little is known about the effects of pollutants on the structure of naturally occurring bacterial communities, particularly under predicted future climate change conditions.

Objectives: Define the bacterial communities associated with different polluted coastal sites, and their interaction with the contaminant pool, including under predicted climate change scenarios. Identify functional genes associated with these communities and taxa indicative of disturbance/pollution. Assess the practical application of high-throughput next generation sequencing techniques as potential indicator systems.

Methods: High-throughput next generation sequencing techniques applied to a combination of field samples and mesocosm experiments are employed to study the effects of various pollutants (e.g. PAHs, organo-phosphates, PCBs, mercury) on naturally occurring bacterial communities from Baltic Sea coastal waters and sediments.

Results: Bacterial taxa within the natural communities responded very differently when exposed to different pollutants. A number of patterns were identified, including some taxa being stimulated by certain pollutants, and others repressed. Considering the decreasing cost of metagenomics analyses, bacterial communities, or specifically chosen taxa, may represent rapid and efficient indicators of environmental disturbance.


Bacterial community composition
as indicator of disturbance
Input of pollutants from
industrial activity and runoff
Selection of bacteria by
toxicity/resistance
Production of toxic metabolites
Release of pollutants from
contaminated sediments

PW065 The effects of selection on ecological dynamics and biofilm characteristics of a multi-species community

Robyn Jerdan[1], Nia White[2], Andrew Spiers[1]

[1]*Abertay University, School of Science, Engineering and Technology, Dundee, United Kingdom*
[2]*Abertay University, Graduate School, Dundee, United Kingdom*

Background: Multi-species biofilms are complex community assemblages central to many ecological processes and are also of growing concern in hospital infections. However, little is known about the establishment, maturation and succession of these ubiquitous structures.

Objectives: To investigate the effects of selection within multi-species biofilm communities and establish the ecological dynamics and subsequent biofilm characteristics over time.

Methods: Utilised a model microcosm system in which the Air-Liquid (A-L) interface biofilms were produced by a soil bacterial community and characterised by total growth (OD_{600}), biofilm strength (g) and attachment levels (crystal-violet A_{570}) measurements. Communities were subjected to serial transfers with different selection regimes including incubation conditions, sample type and transfer times. Biofilm characteristics were monitored after each transfer and to assess the final change in diversity, isolates from both the initial and end communities were compared based on biofilm and phenotypic characteristics. Single isolates from these communities were tested for antagonistic and competitive interactions, and were combined to explore the effect of member numbers on biofilm characteristics.

Results: Preliminary analysis suggests random fluctuations occurred in community biofilm characteristics during the serial transfers, with a shift in phenotypic space and change in diversity occurring with longer incubation periods between transfers. Many community members demonstrated competitive interactions, and a comparison of selected and synthetic communities suggest that optimum total growth and attachment levels are reached with 4 and 8 members, after which competition or exploitation may hinder better growth levels or improved biofilm–formation (i.e. productivity).

PW066 A three-year field fertilisation study on intercrop and monocrop agroecosystem: how do soil microbiome and nitrogen cycle related genes respond?

Honghong Li[1], Petri Penttinen[1], Kristina Lindström[1]

[1]*University of Helsinki, Ecosystems and Environment Research Programme, Helsinki, Finland*

Background: A split-plot design with fertilizer treatment (unfertilized control, organic fertilizer, and synthetic fertilizer) was main plot factor, and crop treatment (fallow, red clover, timothy, and a mixture of red clover and timothy) was sub-plot factor.

Objectives: To study the response of soil microbiome and nitrogen cycle related genes to fertilizer and crop treatments.

Methods: Illumina's sequencing V_3-V_4 of 16S rRNA; qPCR.

Results: During 2014 and 2015, soil bacterial alpha-diversity and microbiome changed (P<0.05). In 2015, the alpha-diversity (richness, diversity, and evenness) was lower in fallow than that in plant treatments (P<0.05). Similarly, microbiome in fallow was different from that in plant treatments (P<0.05); compared to plant treatments, *Actinobacteria, Bacteroidetes*, *Chloroflexi, Verrucomicrobia*, and *Patescibacteria* were less abundant, while *Gemmatimonadetes* and *Planctomycetes* were more abundant in fallow (P<0.05). At the end of the experiment, evenness and microbiome in organic were different from those in control and synthetic treatments (P<0.05); *Proteobacteria* and *Bacteroidetes* were more abundant in organic than in synthetic(P<0.05). Within unfertilized control, alpha diversity was generally highest in mixture, followed by red clover and timothy. The alpha-diversity of plant treatments was higher within organic and lower within synthetic than without fertilization. The microbiome correlated with environmental factors and the abundances of functional genes (P<0.05); pH, moisture, total nitrogen content, and total carbon content were the most important factors. AOB and *nirK* were more abundant in synthetic than in unfertilized control and organic treatments, while AOA and *nirS* were less abundant in mixture than in red clover and timothy treatments (P<0.05).

PW067 Analyses of metagenome and culture collection illustrate anthropogenic impact on the antibiotic resistome and mobilome of Han River, South Korea

Kihyun Lee[1], Dae-wi Kim[1], Do-Hoon Lee[1], Yong-Seok Kim[1], Ji-Hye Bu[1], Ju-Hee Cha[1], Cung Nawl Thawng[1], Hoon-Je Sung[1], Jin Ju Kim[1], Woo Jun Sul [1], Chang-Jun Cha[1]

[1]Chung-Ang University, Department of Systems Biotechnology, Anseong, Korea, Rep. of South

Background: Environmental resistome has been a growing concern in relation to the spread of antibiotic resistance in the clinical settings. It is important to understand how anthropogenic activities influence the resistome and the associated mobilome.

Objectives: To evaluate the impact of modern day selective pressure on shaping the structure of microbial communities and antibiotic resistome, we characterized geographical variations of antibiotic resistome in Han River across the Korean peninsula, which is an ideal aquatic ecosystem with a gradient of anthropogenic activities.

Methods: Both culture-dependent and -independent approaches were employed to explore the resistome and mobilome in Han River from upstream (pristine) to downstream (estuarine) regions at the three different seasons. More than 1800 environmental resisatant bateria were isolated and characterized for resistance phenotypes. Antibiotic resistance genes (ARGs) and mobile genetic elements (MGEs) were analyzed by high-capacity quantitative PCR arrays and metagenome sequencing.

Results: Blooming of ARGs was observed at the populated downstream regions of Han River. Various indicators of anthropogenic loads were correlated with the abundance of ARGs. Rsistome composition was distinct and more diverse at the downstream compared to the upstream, while such a geographic contrast was not evident in the taxonomic composition and overall functions of microbial communities. Analysis of metagenome contigs and culture isolates revealed similar taxonomic snapshots of the river resistome. Comparison of ARG seqeunces revealed that the downstream river resistome was associated with those of human gut and pathogens. Our results provide a fundamental understanding of dynamics of river resistome influenced by anthropogenic acvtivities.

PW068 Effects of carbon content and sulfate fertilization on thioarsenate biogeochemistry and on rhizosphere microbiome in rice paddies

Sarah Zecchin[1], Jiajia Wang[2], Maria Martin[3], Marco Romani[4], Britta Planer-Friedrich[2], Lucia Cavalca[1]

[1]Università degli Studi di Milano, Department of Food, Environmental and Nutritional Sciences (DeFENS), Milano, Italy
[2]Universitaet Bayreuth, Umweltgeochemie, Bayreuth, Germany
[3]Università degli Studi di Torino, Dipartimento di Scienze Agrarie, Forestali e Alimentari (DISAFA), Grugliasco (TO), Italy
[4]Ente Nazionale Risi (ENR), Centro di Ricerche sul Riso, Castello d'Agogna (PV), Italy

Background: Arsenic content in rice grains is limited by European Union directives. In rice paddies, continuous flooding leads to the dissolution of iron (oxyhydr)oxides and release of arsenic from soil minerals with subsequent uptake by rice plant and translocation to the grains. Sulfate fertilization was suggested to reduce arsenic accumulation in rice grains. While direct and indirect microbial arsenic transformations have been widely studied over the last years in rice paddies, the dynamics involving thioarsenates are poorly characterized.

Objectives: Composition and activities of the rhizospheric microbial communities were investigated in relation to carbon content, sulfur fertilization, thioarsenates occurrence in pore-water and arsenic speciation in rice.

Methods: Rice was cultivated in soils with different carbon content and sulfate fertilization in mesocosms placed in the open air. The main physic-chemical parameters were monitored in the pore-water and related to arsenic accumulation in rice grains. Microbial communities were screened by 16S rRNA genes Illumina sequencing in rhizosphere soil and pore water at different time points. Genes related to sulfur and arsenic metabolism were quantified by real-time quantitative PCR.

Results: During rice growing season, sulfate addition led to a higher proportion of thioarsenates and methylated arsenic in the pore-water. In rice grains, total arsenic decreased while the proportion of methylated arsenic increased. Illumina libraries revealed that in rhizosphere soil and in pore-water sulfate-reducing microorganisms were promoted, probably linked to higher arsenic thiolation. In the pore-water of sulfate-fertilized mesocosms, arsenic-methylating microorganisms were more abundant, possibly explaining the higher proportion of methylated arsenic in rice grains.

PW069 Assessment of the potential antimicrobial effect of seagrasses on heterotrophic bacteria in a tropical lagoon

Hana S. Sonbol[1;2], Tamara M. Huete-Stauffer[1], Maria LI. Calleja[1], Carlos M. Duarte[1], Xosé Anxelu G. Morán[1]

[1]King Abdullah University of Science and Technology, Red Sea Research Center (RSRC), Thuwal, Saudi Arabia
[2]Princess Nourah bint Abdulrahman University, Science College, Biology Department, Riyadh, Saudi Arabia

Background: Seagrass meadows are key coastal ecosystems that have been proposed as natural filters for human pathogens.

Objectives: The potential impact of seagrasses (a mixed community of *Cymodocea serrulata* and *Halodule uninervis* plus a monospecific *Enhalus acoroides*) on heterotrophic bacteria was evaluated in a tropical lagoon by comparing samples within and outside the respective meadows (a non-vegetated area 15 m away).

Methods: We analyzed the abundance and cellular characteristics with flow cytometry and the diversity of 16S rRNA gene, together with dissolved organic matter (DOM) fluorescence at ~350 nm (referred to as tryptophan amino-acid peak-T and documented to positively correlate with the abundance of human pathogens).

Results: Our results showed that although bacterial abundances were not significantly different between locations, the contribution of high nucleic acid (HNA) cells and mean cell size were consistently higher within the meadows. Bacterial diversity showed a similar composition in both locations. However, the two most abundant Bacteroidetes genera, with some of their members known to be opportunistic pathogens, were 24% less abundant in the mixed community than outside. Peak-T fluorescence was also reduced at the mixed seagrass meadow and showed a significant and positive correlation with total bacterial numbers, a correlation driven by the samples outside the meadows that showed, on average, higher fluorescence. These results suggest that seagrasses may have positive effects in potentially scavenging pathogenic microbial agents. Whether this is intensified by synergistic strategies in a mixed community meadow or it is driven by a specific seagrass species still needs to be explored.

PW070 The magnetosomes as protective shields against metal stress in Magnetospirillum gryphiswaldense

David Muñoz Rodríguez[1;2], Lourdes Marcano[1], Rosa Martín-Rodríguez[3], Ana García Prieto[4;5], M.Luisa Fdez-Gubieda[1;5], Alicia Muela[2;5]

[1]*Universidad del País Vasco, Electricity and Electronics, Leioa, Spain*
[2]*Universidad del País Vasco, Immunology, Microbiology and Parasitology, Leioa, Spain*
[3]*Universidad de Cantabria, QUIPRE , Santander, Spain*
[4]*Universidad del País Vasco, Applied Physics I, Bilbao, Spain*
[5]*Basque Center for Materials, Applications and Nanostructures, Leioa, Spain*

Background: Magnetotactic bacteria are a group of aquatic prokaryotes capable of navigating along the Earth´s magnetic field lines thanks to the synthesis of a chain of membrane-enclosed magnetic nanoparticles called magnetosomes. According to the most accepted hypothesis, the only purpose of producing magnetosomes would be to provide the bacteria a more efficient movement in a chemical redox gradient within the water column, in order to reach optimal conditions for their growth. However, the magnetosomes could have other physiological roles.

Objectives: Analyse the role of magnetosomes in the tolerance of certain metals in *Magnetospirillum gryphiswaldense.*

Methods: We perform the tolerance curves of *M. gryphiswaldense* MSR-1 to five transition metals (Co, Mn, Ni, Zn and Cu), growing the bacteria with different concentrations of each metal in the culture medium, either when the bacteria present magnetosomes or not. In addition, we analyse by means of X-ray absorption spectroscopy (XANES) the incorporation of each metal in the cell, as well as in the magnetosome structure.

Results: We have observed that the tolerance to all metals tested is significantly higher (up to four-fold higher in the case of Mn) when the bacteria present magnetosomes. Furthermore, the spectroscopic analysis revealed that *M. gryphiswaldense* stores Co, Mn, Zn and Cu when there is a metal excess in the medium. While Co and Mn are incorporated in the mineral core of the magnetosomes, that is not the case of Zn and Cu.

PW071 Biofilm formation vs stress factors: who can play at the game

Olga Iungin[1], Oleksandr Tashyrev[1], Andrew Spiers[2]

[1]*Zabolotny Institute of Microbiology and Virology of the NAS of Ukraine, Kiev, Ukraine*
[2]*Abertay University, Dundee, United Kingdom*

Background: The use of different chemicals for agriculture, industry and mining has caused pollution of agrarian soils which provokes changes in rhizosphere microflora. We have studied 10 bacterial strains isolated from a winter wheat Cd-polluted field in Ukraine by their taxonomic position, biochemical properties and resistance to 3 classes of toxicants: heavy metals (Cu^{2+}, Cd^{2+}), non-metals (perchlorate-ion), organic xenobiotic (1-chloro-4-nitrobenzene, CNB).

Objectives: To study the effect of toxicants on the biofilm–formation ability of individual strains and a mixed community.

Methods: Biofilm characteristics (total microcosm growth, biofilm strength and attachment to the microcosm walls) were studied by combined biofilm assay (n = 4) with four treatments including 100 mg/L Cu^{2+}, 25 mg/L Cd^{2+}, 300 mg/L ClO_4^-, and 100 mg/L CNB, with correlations and Principal component analysis (PCA) used to investigate data.

Results: We found that microbial community had a greater resistance to the toxicants compare to individual strains. The presence of heavy metals increased the strength of biofilms, and in most cases growth and biofilm strength were positively correlated. However, in the presence of Cd^{2+} this correlation was lost. Perchlorate affected bacteria, increasing mucus production and biofilm strength though it also reduced attachment levels. Finally, we also found that CNB could be used as a source of Carbon and energy during biofilm–formation.

PW072 spatiotemporal microbial community analysis of the Nile river along the Cairo urban metropolis

walaa eraqi[1], marwa elrakaiby[1], salwa megahed[1;2], noha youssef[3], mostafa elshahed[3], Aymen Yassin[1]

[1]Faculty of Pharmacy, Cairo University, Microbiology and Immunology, Cairo, Egypt
[2]Faculty of Pharmacy, October University for Modern Sciences and Arts (MSA), Microbiology and Immunology, Giza, Egypt
[3]Oklahoma State University, Microbiology and Molecular Genetics, Stillwater, United States

Background: Fresh water systems need microbial community analysis to monitor the influence of xenobiotic and anthropogenic inputs particularly in urban and industrial settings. The Nile river is one of the longest rivers in the world, located in northeastern Africa and spanning eleven countries. For Egypt in particular, it is the primary and main water source. The Nile river runs through Cairo, the capital of Egypt with over 20 million inhabitants, and is consequently subjected to an enormous burden of inner-city environmental influences. It is essential to monitor how the microbial community in and around such a dynamic setting is evolving.

Objectives: Identification of the microbial community structure of both the Nile river surface water and river sediments along the path of the natural flow of the river through the Cairo area metropolis in two different seasons, in an attempt to link such community dynamics to the various environmental influences that might contribute to its composition and development.

Methods: Surface water samples and sediments from the river banks were collected from five different locations that span the flow of the river from entrance to exit and passing by both residential and industrial areas. Next generation sequencing was used to analyze the 16S rRNA gene composition followed by appropriate computational analysis.

Results: Considerable stability in water microbial community structure composition was revealed despite some minor seasonal differences on the phylum level. Similarity was observed in comparison with other global fresh water systems. Soil sediments samples had a more complex and diverse composition.

PW073 Genomic characteristics of Desulfonema ishimotonii Tokyo01 implying horizontal gene transfter among phylogenetically dispersed gliding bacteria

Miho Watanabe[1], Hisaya Kojima[1], Kazuhiro Umezawa[1], Manabu Fukui[1]

[1]*The Institute of Low Temperature Science, Hokkaido University, Sapporo, Japan*

Background: The genus *Desulfonema* encompasses mesophilic filamentous sulfate-reducing bacteria which have gliding motility. Previous studies have reported physical association implying *Desulfonema* species have close relationships with filamentous sulfur oxidizers and cyanobacteria.

Objectives: We describe the complete genome sequence of *D. ishimotonii* Tokyo 01^T. The sequenced genome was analyzed with a focus on genetic transfer among different lineages.

Methods: Genome sequencing was carried out using the PacBio RS II platform. All putative protein sequences were searched against the NCBI-nr database to evaluate the phylogenetic affiliation. REBASE database was utilized to identify the DNA restriction-modification system. For a comparative analysis, COGs annotations of four closely related organisms were retrieved from the IMG/M database.

Results: Genome analysis showed that more than 20% of predicted proteins are putatively of foreign origins. Many of these proteins had the highest sequence identities with proteins encoded in genomes of filamentous bacteria, including giant sulfur oxidizers, cyanobacteria and bacteria of the candidate phylum KSB3. In the genome of strain Tokyo 01^T, genes involved in the DNA recombination and repair were enriched in comparison to the closest relatives. Some of these genes and putative inteins in DnaE and GyrB were also related to those of organisms outside of the class *Deltaproteobacteria*. The genomic data suggested significant genetic sharing among filamentous gliding bacteria in phylogenetically dispersed lineages, including *Desulfonema* species. This study provides insights into the genomic evolution of filamentous bacteria belonging to diverse lineages, characterized by various physiological functions and different ecological roles.

PW074 Adaptation of the psychrotolerant sulfate-reducing bacterium 'Desulfovibrio gilichinsky' K3S to the grow at subzero temperatures.

Yana Ryzhmanova[1], Viktoria Shcherbakova[2]

[1]Skryabin Institute of Biochemistry and Physiology of Microorganisms, Pushchino Center for Biological Research of the Russian Academy of Sciences, Pushchino, Moscow region, Russian Federation
[2]Skryabin Institute of Biochemistry and Physiology of Microorganisms, Federal Research Center, Pushchino Center for Biological Research of the Russian Academy of Sciences, Pushchino, Moscow region, Russian Federation

Background: Cryopegs are ancient aquatic ecosystems which characterized by permanently subzero temperatures and high-level total salinity. Recently novel sulfate-reducing bacterium '*Desulfovibrio gilichinskyi*' K3S^T was isolated from a Yamal Peninsula cryopeg. Strain K3S^T demonstrated growth at subzero temperatures and apparently took part in the terminal stage of organic matter decomposition in the cryopeg. Therefore, the bacterium should to develop some protective mechanisms for survival in these harsh conditions.

Objectives: to identify the strategies for cold adaptation of '*D. gilichinskyi*' K3S^T.

Methods: The whole-genome sequence of strain K3S^T was conducted by the DOE Joint Genome Institute by using Illumina HiSeq 2500-1TB sequencing method. Draft genome sequence was deposited in JGI under number IMG ID 2708742538, and listed in SRA as ERS1894632. Cellular fatty acid (CFA) profile was determined by GC-MS of methyl ester derivatives.

Results: The CFA analysis showed that cells of strain K3S^T contain high level of the polyunsaturated fatty acids (59.8%), including iso/anteiso-branched fatty acids (20.6%). Moreover, genome data analysis showed presence fatty acid desaturase gene, which allows to increase the ratio of unsaturated fatty acids and, consequently, the membrane fluidity. Also genome analysis showed that strain K3S^T uptakes glycine betaine and synthesizes trehalose, which are the most common microbial osmo-/cryoprotective compounds. Changing in the amino acid composition was also observed: decline in the number of Arg, Gln, Ala and Pro, with an increase amount of hydrophobic residues Leu and Phe, relative to mesophiles. All these changes allow increase membrane fluidity at low temperature and protect bacterium from freezing damage.

PW075 Genetic background of close relationship between methanogenic archaeon Methanosarcina mazei JL01 and bacterium Sphaerochaeta associata JLS2 from Arctic permafrost

Viktoriia Oshurkova[1], Olga Bochkareva[2], Olga Troshina[1], Viktoria Shcherbakova[1]

[1]*Skryabin Institute of Biochemistry and Physiology of Microorganisms, Federal Research Center "Pushchino Center for Biological Research of the Russian Academy of Sciences", Pushchino, Russian Federation*
[2]*Skolkovo Institute of Science and Technology, Moscow, Russian Federation*

Background: Polar permafrost appears to be phylogenetically complex deep biosphere. Only a few studies have enriched the native methane-producing archaea that might provide positive feedbacks to climate change. Due to low energy benefit of the last stage of mineralization of organic compounds, methanogens have to be involved in anaerobic syntrophic interactions with other microorganisms. The binary methanogenic culture JL01 consisting of methanogenic strain *Methanosarcina mazei* sp. JL01 and novel anaerobic saccharolytic bacteria *Sphaerochaeta associata* GLS2^T was isolated from Holocene permafrost (Kolyma lowland, Russia) after long-term cultivation at 15°C.

Objectives: determination the role of close association between methanogenic archaea and saccharolytic bacteria, obtained from Arctic permafrost, based on comparative analysis of complete genomes.

Methods: Hungate anaerobic technique; 16S rRNA gene clone library, PCR and sequencing; genome annotation

Results: New psychroactive methanogenic strain *Methanosarcina mazei* JL01 and novel anaerobic saccharolytic bacteria *S. associata* GLS2^T were isolated and characterized. Genome analysis of *Sphaerochaeta* spp. revealed the absence of genes encoding proteins necessary in the last stages of peptidoglycan synthesis, but large number of polysacharides, amino acids, peptides transport genes and a complete set of metabolic pathways (glycolysis, pentose phosphate cycle, fermentation) genes which let assume the ability of the strain GLS2^T to use polysaccharide components of methanogen's cell wall. Also it was shown the 25% increase of methane production as a result of methanosarcina and bacterium *S. associata* JLS2^T co-cultivation which indicates that methanogenic strain JL01 may utilize acetate, produced by *Sphaerochaeta associata* GLS2^T.

PW076 Novel strategy for studying dissemination of mobile genetic elements in the gut microbiome

Mariajose Garzon[1], Beatriz Herrera[1], Asmus Kalckar Olesen[2], Rafael Pinilla[2], Søren Johannes Sørensen[2], Carles Ubeda[1;3]

[1]Fisabio, Department of genomics and health, València, Spain
[2] University of Copenhagen, Department of Biology , Copenhagen, Denmark
[3]CIBER - Center for Biomedical Research Network, Epidemiología y Salud Pública, Madrid, Spain

Background: Antibiotic resistant pathogens (ARPs) represent a real threat for life. Antibiotic resistant genes are frequently encoded in mobile genetic elements (MGE) that facilitate their dissemination. In the gut, ARPs contact with commensal bacterial species that may facilitate the flow of MGE between pathogenic and commensal species.

Objectives: Develop a new methodology to study dissemination of MGE among commensal bacteria in the gut.

Methods: We have adapted a previously described methodology (Klümper, U. et al., 2015, ISME J.) based on fluorescent activated cell sorting (FACS) and next-generation sequencing technologies, in order to study MGE dissemination in the anaerobic environment of the gut. Briefly, the superfolder green fluorescent protein gene (sfGFP), under the control of a tetracycline inducible promoter, was introduced in the conjugative plasmids R388 and RP4. These constructions were introduced in an *Escherichia coli* strain expressing the Tet repressor (TetR). The generated strain was used as donor in conjugation experiments under anaerobic conditions.

Results: Transconjugants (i.e. GFP fluorescent cells) could be detected after incubation of donor bacteria with: *E. coli* not expressing TetR (used as positive control) or a culture of murine caecal contents. No fluorescent bacteria could be identified in pure cultures of donor or recipient bacteria. The number of sorted transconjugants (>40000 cells) was sufficient to perform 16s rRNA sequencing (currently undergoing analysis to identify the taxonomy of recipient bacteria).

In conclusion, we have developed a new method to study MGE transfer among intestinal bacteria in anaerobic conditions.

PW077 Diversity and metabolic functions of diazotrophic microorganisms in the largest European lagoon

Rūta Stanislauskienė[1], Aurelija Samuiliovienė[2], Rolandas Meškys[1], Marco Ventura[3], Maren Voss[4], Mindaugas Žilius[2]

[1]*Vilnius University Life Sciences Center Institute of Biochemistry, Department of Molecular microbiology and biotechonology, Vilnius, Lithuania*
[2]*Marine Research Institute of Klaipeda University, Klaipėda, Lithuania*
[3]*University of Parma, Department of Chemistry, Life Sciences and Environmental Sustainability, Parma, Italy*
[4]*Leibniz Institute for Baltic Sea Research, Department of Biological Oceanography, Rostock, Germany*

Background: The temperate estuarine systems are characterized by seasonal nitrogen (N) limitation, which creates favourable conditions for diazotrophs. Heterocystous cyanobacteria are currently believed to fix atmospheric dinitrogen (N_2), and therefore can resist N limitation. Large colonies of heterocystous cyanobacteria can be accompanied by pico-cyanobacteria, which are assumed to be important putative heterotrophic diazotrophs in estuarine environments. However, biodiversity and role of diazotrophic community in the lagoon functioning remains still poorly understood.

Objectives: To identify the diversity and temporal dynamic of pelagic microbial community of lagoon by determining their metabolic capacity in N_2-fixation.

Methods: Community DNA was extracted using QIAamp Fast DNA Stool Mini Kit (Qiagen) from water samples collected from April to November 2018 in representative areas of the Curonian Lagoon. Sequencing of 16S rRNA was performed with at least 30 000 reads per sample using an Illumina platform (Miseq). Shotgun metagenomics analyses were conducted with up to 25 million of reads per sample using NextSeq (Illumina). Taxonomic classification was performed based on SILVA and NCBI RefSeq database. Functional analysis of the reads was conducted based on the EggNog and MetaCyc database. Rates of pelagic N_2-fixation were measured by $^{15}N_2$ incorporation technique, analysing samples with EA-IRMS.

Results: We determined the taxonomic diversity and metabolic functions of bacterial community in water column of lagoon. We also examined how seasonal shift from N excess to its limitation affects the community structure of diazotrophs and how these changes alter metabolic capacity to fix N_2.

PW078 Novel thermophilic archaea from Kamchatka hot springs: ecology and metabolism

Anna Perevalova[1], Alexander Merkel[1], Stepan Toshchakov[1], Elizaveta Bonch-Osmolovskaya[1]

[1]Research Center of Biotechnology Russian Academy of Sciences, metabolism of extremophilic prokaryotes, Moscow, Russian Federation

Background: A great number of uncultured archaea have been detected with molecular techniques both in non-thermal environments and in hot habitats (e.g., Hug et al., 2016). However, the majority of novel archaeal lineages remain elusive: no cultivated strains are still available. Recent advances in metagenomic techniques gave us some hints on the metabolism of uncultured archaeal lineages from complex environments (e.g., Zhou et al., 2018; Liu et al., 2018).

Objectives: Kamchatka hot springs with different temperatures and pH of water.

Methods: The hot springs were analyzed using high-throughput sequencing methods and cultivation approach.

Results: Many uncultured archaeal lineages were detected, among them uncultured *Thermoplasmatales*. In some of studied hot springs the number of representatives of the group was rather high (up to 30%). In order to get some information on the metabolic properties of the deep-branching group, we made an attempt to obtain its representatives in laboratory cultures. We succeeded in obtaining several sustainable enrichments of uncultured *Thermoplasmatales* group growing at 55-60°C. These results help us to through some light on the metabolism of the novel thermophilic archaea.

This work was supported by Russian Science Foundation grant №17-74-30025.

[1]. Hug, L.A., B.J. Baker et al., and J.F. Banfield. 2016. Nature Microbiology Letters 16048.

[2]. Zhou Z, Liu Y, Lloyd KG, Pan J, Yang Y, Gu JD, Li M. ISME J. 2018 Dec 4.

[3]. Liu Y, Zhou Z, Pan J, Baker BJ, Gu JD, Li M. ISME J. 2018 Apr;12(4):1021-1031.

PW079 Detection of a qnrD2 plasmid isolated from M.morganii of systematically diseased cold-blooded amphibians

Katharina Jurascheck[1], Claudia Jäckel[1], Silvia Schmoger[1], Christina Banyai[1], Tanja Skladnikiewicz-Ziemer[1], Britta Lesniewsky[1], Karsten Nöckler[1], Annemarie Käsbohrer[1], Jens André Hammerl[1]

[1]*German Federal Institute for Risk Assessment, Department of Biological Safety, Berlin, Germany*

Background: Reports on the emergence and occurrence of fluoroquinolone/quinolone resistance determinants point out a steadily increasing risk for public health. The most common cause for quinolone resistances are point mutations in the DNA gyrase and topoisomerase. However, several plasmid-mediated resistance factors were reported to be involved in the occurrence of quinolone resistant bacteria.

Objectives: This study was initiated to compare stability, antimicrobial susceptibility and the genetic basis of *qnrD* plasmids from *M. morganii* comprising different pentapeptide repeat protein D subclones (QnrD1/D2).

Methods: Bacteriological investigation of systemic diseased African bullfrogs from Tanzania resulting in the isolation of four *M. morganii* isolates exhibiting reduced susceptibility to fluoroquinolones and nalidixic acid by antimicrobial susceptibility testing. Molecular analysis indicated the presence of small *qnrD* plasmids. By Sanger sequencing the genetic basis of the plasmids was determined and analyzed by bioinformatics. Furthermore, the plasmid stability and the resistance behavior of the plasmids were compared in different enterobacteria.

Results: Plasmid profiling revealed the presence of a previously described 2.7 kb plasmid in three and a novel 1.9 kb plasmid in one the investigated isolates. Sequence analysis showed, that the smaller plasmid encodes a *qnrD2* gene. In contrast to the predominant *qnrD* plasmid prototype, microbiological analyses showed that the replication stability of pMM8916 seems to be limited over long-time periods under nonselective conditions among various genera of the Enterobacteriaceae. This study disclosed the existence of a new *qnrD2 M. morganii* plasmid, which is, to our knowledge, the smallest described so far.

PW080 Cultural Heritage-Biome: dissection of the microbial community to contrast Florence Cathedral biodeterioration

Alice Checcucci[1], Beatrice Agostini[2], Alba Patrizia Santo[3], Brunella Perito[1]

[1]*University of Florence, Biology, Italy*
[2]*Opera di Santa Maria del Fiore*
[3]*University of Florence, Department of Earth Sciences*

Background: Stone-built cultural heritage (CH) exposed to urban environment represent a habitat where heterogeneous microbial communities can grow inducing significant biodeterioration. An in-depth investigation of microbial community composition and its metabolic potential is essential to take the appropriate measures to control its growth. However, metagenomic approaches are still few applied in biodeterioration studies.

The Cathedral of Santa Maria del Fiore (SMFC) is a World Heritage site in Florence (IT), and its conservation is a main issue of worldwide concern. It is covered by polychrome stone panels which show, in some extended areas, patinas and discoloration due to microbiological growth.

Objectives: The aim of this work was the investigation of the state of conservation of SMFC marbles and the microbial community involved in their deterioration. This information will be used for planning field tests with innovative and specific biocides to contrast biodeterioration.

Methods: Sampling from patinas of selected marble areas and *in-situ* microscopy was performed. Bacterial and fungal communities' composition by using 16S and ITS rDNA metagenomics sequencing was analyzed. Field trials with innovative low environmental impact biocides will set up and community abundance variation will be monitored over time.

Results: For the first time, this work provides details on the microbial community's composition involved in SMFC marble deterioration. The work will provide information on the effectiveness of specific biocide treatments on marble microbial growth and new insights for the selection of monument-specific biocides through metagenomic analysis.

PW081 Genetic variability and antimicrobial resistance of Klebsiella spp. isolates from sewage water of slaughterhouses

Michael Savin[1], Claudia Jäckel[2], Silvia Schmoger[2], Marijo Parcina[1], Judith Kreyenschmidt[1], Annemarie Käsbohrer[2], Jens André Hammerl[2]

[1]University of Bonn, Faculty of Agriculture, Institute of Animal Sciences, Bonn, Germany
[2]German Federal Institute for Risk Assessment, Department of Biological Safety, Berlin, Germany

Background: *Klebsiella* spp. are Gram-negative opportunistic pathogens prevalent on plants, in water and soil but also colonizing a wide range of livestock/wildlife animals. Klebsiellae were recognized as an important threat to public health due to their high level of antimicrobial resistance, mainly associated with the presence of mobile genetic elements.

Objectives: In this study, ESBL-producing *Klebsiella* spp. isolates from sewage water of poultry and swine slaughterhouses were characterized by whole genome sequencing and antimicrobial resistance testing.

Methods: Antimicrobial susceptibility testing of *Klebsiella* spp. isolates was performed using broth microdilution following CLSI guidelines and EUCAST epidemiological cut-off values. Whole-genome sequencing and bioinformatics were performed to reveal the genetic basis of the observed resistances and the diversity of the isolates in different processing stages of slaughtering.

Results: Bioinformatic analyses revealed that prevailing isolates represented a high genetic diversity in its MLST-type and virulence profile. Overall, isolates exhibited many plasmid sequences, that carried various antimicrobial resistances. The majority of the isolates harbor *bla*$_{SHV}$ genes causing its ESBL-phenotype. Some of them also comprise determinants involved in the development of a carbapenem-resistance phenotype. The genetic basis of the isolates, their antimicrobial resistances and the content of mobile genetic elements will be presented in detail. Our study confirms that characterized klebsiellae comprises diverse mobile genetic elements that may important vectors for the transmission of antimicrobial resistances. Some of the plasmids are closely related to plasmids of clinical *Klebsiella* isolates from humans. However, to date their impact on human health is unknown and need to be assessed.

PW082 Interactive Effects of Ultraviolet Radiation and Temperature on Bacterial Production at the Lirima Thermal Springs in the Chilean Altiplano

Wade Jeffrey[1], Erika Neat[1], Cristina Dorador[2], Martha Hengst[3]

[1]University of West Florida, Center for Environmental Diagnostics and Bioremediation, Pensacola, United States
[2]Universidad Antofagasta, Laboratorio de complejidad microbiana, Antofagasta, Chile
[3]Universidad Católica del Norte, Departamento de Ciencias Farmacéuticas, Antofagasta, Chile

Background: The interaction between environmental conditions in extreme environments presents challenging conditions for microbial growth. There is some evidence that sensitivity to solar UVR is inversely related to temperature, although extensive studies are limited. The Lirima hot springs in the Chilean Altiplano are located at approximately 4000 m above sea level and have extremely high solar irradiance. They are characterized by hot underground freshwaters, enriched in sulfur compounds with pH values from neutral to acidic. Temperatures range between 30 – 80 degrees C. Microbial diversity studies are limited and no measures of in situ growth have been reported.

Objectives: Using the naturally occuring temperature gradient found between the different springs, our goal was to examine the interactions between temperature and solar ultraviolet radiation on bacterial production and compare them to other available data.

Methods: Bacterial production (^{3}H-leucine incorporation) was measured in situ at fifteen different locations within the Lirima springs area over two consecutive days. Weather patterns were similar and both days had clear skies. Temperatures for the incubations ranged between 21 and 74 degrees. At each location production was determined with exposure to full sun, with UVB blocked, with PAR only (UVB and UVA blocked) and in darkness. Four hour incubations were centered around solar noon. Additional measues included pH, conductivity, and microbial diversity (16S rDNA).

Results: Solar radiation in this region is some of the highest ever recorded. Solar effects on bacterial production and microbial diversity data are currently being analyzed and will be discussed with the temperature gradient.

PW083 Impact of the quorum sensing molecule JAI-1 and the Typ VI secretion system of Janthinobacterium in biofilm formation and fungal cell death

Christel Vollstedt[1;1], Laura Nissen[1], Wolfgang R. Streit[1]

[1]*University of Hamburg, Department of Microbiology and Biotechnology, Hamburg, Germany*

Background: Janthinobacterium spp. and bacteria affiliated with the genus Duganella are Gram-negative, motile, aerobic and purple-pigmented bacteria, which are commonly isolated from soil and aquatic samples. While Janthinobacterium as well as Duganella appear to be non-pathogenic to humans, animals, and plants, they are well-known for their antifungal effects. In coincubation assays with different janthinobacterial strains and *F. graminerarum* we observed a biofilm formation around the fungal hyphae leading to a reduced fungal growth.

Objectives: Identification of molecules and mechanisms that control the interaction between bacteria and fungi.

Methods: Genome sequencing of 11 bacteria affiliated with the genera of Janthinobacterium and Duganella. RNA-sequencing of two different strains and mutants. Generation of deletion mutants.

Results: Sequencing data showed that almost all organisms encode for the Janthinobacterium quorum sensing system (JQS) which uses alpha-hydroxyketone-like autoinducer molecules for bacterial communication, named JAI-1. For two strains (J.sp.HH01 and HH102) deletion mutants in the autoinducer synthase were generated. The RNA seq studies of these two isolates and their corresponding JAI-1 deletion mutants showed that the most strongly regulated genes were secondary metabolites and T6SS gene clusters. Interestingly, the T6SS seems to be controlled by the autoinducer JAI-1 within the strain HH102, whereas the strain HH01 did not regulate the T6SS gene expression via JAI-I. Due to this fact, we deleted essential genes in the T6SS in J.sp.HH102 and the corresponding JAI-1 deficient strain to analyse the influence on the fungus.

PW084 Mycorrhiza-assisted phytomanagement of contaminated soil using poplar trees and alfalfa

June Hidalgo[1], Mikel Anza[1], Lur Epelde[1], Julen Urra[1], José M. Becerril[2], Carlos Garbisu[1]

[1]Neiker-Tecnalia, Conservation of Natural Resources, Soil Microbial Ecology Group, Derio, Spain
[2]Universidad del País Vasco (UPV/EHU), Bilbao, Spain

Background: Soil contamination with organic pollutants raises concerns due to their impact on environmental and human health. Our study area, located in one of the most heavily industrialized areas of the Basque Country (Spain), is characterized by the presence of high concentrations of total petroleum hydrocarbons (TPHs) and polycyclic aromatic hydrocarbons (PAHs). In this area, phytomanagement has been proposed as a suitable strategy that combines sustainable site management with gentle remediation options leading to a reduction in pollutant linkages alongside the restoration and/or generation of wider site services. This work was funded by the NANORRIZORREM-2 project (AGL2016-76592-R).

Objectives: The aim of this study was to determine the best phytomanagement strategy for the recovery of soil heavily polluted with a mixture of TPHs and PAHs.

Methods: We carried out an experiment to assess the effects of the inoculation of mycorrhiza and the application of organic amendments on the effectiveness of phytomanagement using poplar trees and alfalfa. The following parameters were measured as indicators of the recovery of soil health: TPHs and PAHs concentration, microbial biomass carbon, total bacteria and fungi, amplicon sequencing (16S, 18S), mineralizable nitrogen, enzyme activities (dehydrogenase, β-glucosidase, β-glucosaminidase, arylsulphatase, xylosidase, phosphatase, Leu-aminopeptidase), respiration, C:N ratio, water soluble organic carbon, cation-exchange capacity, etc.

Results: We observed a phytomanagement-induced recovery of soil health, especially in response to the application of organic amendments that led to a stimulation of microbial biomass and activity. Plant growth was stimulated by the applied treatments.

PW085 Pistacia vera L. oleoresin and levofloxacin: an innovative strategy to tackle the antibiotic resistance in Helicobacter pylori strains

Silvia Di Lodovico[1], Paola Di Fermo[1], Emanuela Di Campli[2], Simonetta D'Ercole[2], Celeste Nicoletta Viscio[3], Antonia Nostro[4], Emanuela Marini[5], Mara Di Giulio[3], Luigina Cellini[3]

[1]*"G. d'Annunzio" University Chieti-Pescara, Department of Pharmacy, Chieti, Italy*
[2]*University of Chieti , Department of Pharmacy, Chieti, Italy*
[2]*University of Chieti , Department of Pharmacy, Chieti, Italy*
[4]*University of Messina, Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, Messina, Italy*
[5]*Polytechnic University of Marche, Ancona, Unit of Microbiology, Department of Biomedical Sciences and Public Health, Ancona, Italy*

Background: The increase of antimicrobial resistance in *H. pylori,* also correlated to its biofilm-forming ability, is often reason of the therapies failure. This phenomen, strongly underlines the need to the search of novel strategies to improve the eradication rate. Natural compounds are proposed as antibiotic-resistant-breakers capable to restore the drugs efficacy.

Objectives: The aim of this work was to evaluate, *in vitro* and *in vivo,* the capability of the *P. vera* L. oleoresin-(ORS) to synergize with levofloxacin-(LVX) against resistant *H. pylori* strains.

Methods: The antimicrobial activity of ORS and LVX and their combinations was determined against a total of 32 *H. pylori* strains by MIC/MBC (in neutral and acidic environments) and checkerboard tests. The anti-biofilm effect was determined by biomass quantification. *In vivo Galleria mellonella* model was used to confirm the *in vitro* data.

Results: Oleoresin and LVX MICs ranged from 780 to 3120 mg/l and from 0.12 to 1.00 mg/l, respectively. MBCs were similar to MICs. Oleoresin was able to synergize with LVX restoring its effectiveness in all resistant detected strains with FIC Index from 0.18 to 0.50. Oleoresin, LVX and their synergistic combinations displayed significant microbial biofilm reductions up to 60.45% at 1/2 MIC. Oleoresin alone and combined with LVX showed a significant protective effect against *H. pylori* infection with a 75% of *G. mellonella* larvae survival.
Oleoresin can be considered a promising potentiator to restore the effectiveness of LVX. Oleoresin plus LVX is an innovative strategy to tackle the *H. pylori* antibiotic resistance phenomenon.

PW086 Phosphomonoesterase activity and bacterial phosphomonoesterase gene abundance in two rivers from southern Chile

Marco Campos[1;2], Joaquin Rilling[1;2], Jacqueline Acuña[1;2], Milko Jorquera[1;2]

[1]Applied Microbial Ecology Laboratory (EMAlab), Department of Chemical Sciences and Natural Resources, Temuco, Chile
[2]Scientific and Technological Bioresource Nucleus (BIOREN), Department of Chemical Sciences and Natural Resources, Temuco, Chile

Background: Phosphorus (P) is an essential nutrient for life, but is also categorized as a pollutant because is constantly deposited in water body sediments by human action, contributing to the eutrophication and frequency of harmful algae blooms. In aquatic environments, P recycling is governed by several abiotic and biotic factors, being key the activity of bacterial enzymes (such as phosphomonoesterases [Pases]). In this context, rivers are important "ecosystem services" in southern Chile, but scarcely studied, particularly in P recycling regulated by bacterial activity.

Objectives: To determine phosphomonoesterase activity and bacterial phosphomonoestase gene abundance in two rivers from southern Chile.

Methods: Superficial sediment samples were taken in triplicate from four anthropized points of Imperial and Toltén rivers during winter 2018. Nutrient contents (C, N and P), enzymatic activity (alkaline (AKP) and acidic [AP] Pases) and bacterial gene abundance (total bacterial [16S rDNA], alkaline [*pho*D and *pho*X] and acidic [*pho*C] Pases) were measured. Then, data were collected and subjected to principal component analysis (PCA) and ANOVA parametric test ($p<0.05$).

Results: PCA analysis showed a clear separations between both rivers, where the higher values of P contents, Pase activity and Pase genes were positively correlated with Imperial river. Interestingly, in general the highest values were found in the coast closest sites with significant statistical differences respect to the coast farthest ones. Our results showed significant differences between both rivers and along their courses. However, deeper studies are required to confirm these observations.
Acknowledgment to the FONDECYT N°3180198 (postdoctoral), N°1160302 and N°11160112 projects.

PW087 Polyhydroxyalkanoate (PHA) producing bacterium using crude glycerol and expired shortening as carbon sources

Kamontam Umsakul[1], Sirinut Duangsook[2], Mananya Martla[1], Nattapon Thongwong[1]

[1]*Prince of Songkla University , Department of Microbiology, Songkhla, Thailand*
[2]*Prince of Songkla university, Department of Microbiology, Songkhla, Thailand*

Background: Polyhydroxyalkanoates (PHAs) are produced by various microorganisms as a source of carbon and energy storage. They attract much attention since they are fully biodegradable and biocompatible but the production cost is still expensive.

Objectives: Polyhydroxyalkanoate (PHA) production by a potential soil bacterium 8G1, isolated from Pru-Dang forest, Narrativas, Thailand, was investigated.

Methods: Two cheap carbon sources, crude glycerol and expired shortening were used as carbon sources to minimize the PHA production cost. PHA was extracted using both chemical and biological methods. In this study, we report on the use of mealworm (*Tenebrio molitor*) to recover PHA granules from 8G1 cells.

Results: Expired shortening gave a higher PHA production and better cell growth. When cultured in mineral salt medium containing crude glycerol as a carbon source, 8G1 produced the highest quantity of PHA of 72 % /cell dry weight at 96 h. While cultured with expired shortening, 8G1 produced the highest quantity of PHA of 92 %/ cell dry weight at the much shorter time of 60 h. Biological recovery potentially reduces the use of solvents and chemicals. The weight average molecular weight (M_w), number average molecular weight (M_n), of the chloroform extracted and biologically recovered and purified PHA granules were not much different. These results indicated that the mealworm extraction method could be used to replace the solvent extraction of polymer.

PW088 Novel antibiotic resistance genes from Stenotrophomonas maltophilia and Chryseobacterium sp. of soil origin discovered by functional gene library

Julija Armalyte[1], Laurita Klimkaite[1], Ignas Ragaisis[1], Renatas Krasauskas[1], Jurate Skerniskyte[1], Modestas Ruzauskas[2], Edita Suziedeliene[1]

[1]*Vilnius University, Institute of Biosciences, Life Sciences Centre, Vilnius, Lithuania*
[2]*Lithuanian University of Health Sciences, Microbiology and Virology Institute, Kaunas, Lithuania*

Background: As the most diverse habitat of microorganisms, soil has also been recognised as a reservoir of both antibiotics and the antibiotic resistance genes (ARGs). The bacteria naturally inhabiting the soil or water often possess the innate ARGs to counteract the chemical compounds produced by competitors living in the same environment. *Stenotrophomonas maltophilia* is an ubiquitous environmental bacterium, also associated with an increasing number of nosocomial infections. *Chryseobacterium* sp. found primarily in soil and water and is increasingly found to colonize the immunocompromised patients through contaminated medical devices and liquids. Both bacteria have high innate resistance to antibiotics, which complicates the treatment of their infections.

Objectives: We have recently collected antibiotic resistant bacteria from agricultural soils, *Stenotrophomonas* sp. and *Chryseobacterium* sp. being among the most antibiotic resistant bacteria selected. We aimed to select the ARGs responsible for their innate resistance by generating and screening functional gene libraries.

Methods: Genomic DNA was isolated from *S. maltophilia* and *Chryseobacterium* sp. resistant to at least 2 classes of antibiotics. gDNA was fragmented and used to create functional DNA fragment libraries, which were transformed to *E. coli* and screened for antibiotic (beta-lactams, aminoglycosides, quinolones) resistant clones.

Results: During the screening of functional gene libraries of two soil bacteria we were able to find novel variants of aminoglycoside and beta lactam ARGs. Two beta lactamase encoding genes were isolated from *Chryseobacterium* sp. functional gene library, one of them being a variant of IND-like metallo-beta-lactamase.

PW089 Development of a risk-based approach for the identification of environmental reservoirs of Mycobacterium avium subspecies paratuberculosis (Map) in cattle production systems

Eulyn Pagaling[1], Lisa Avery[2], Geoff Elliot[2], Malcolm Coull[3], Charlie Shand[2], David Lumsdon[2], Renate Wendler[2], Nadine Thomas[1], Lucinda Robinson[1], Colin Campbell[1], Rupert Hough[3]

[1]*The James Hutton Institute, Ecological Sciences, Aberdeen, United Kingdom*
[2]*The James Hutton Institute, Environmental and Biochemical Sciences, Aberdeen, United Kingdom*
[3]*The James Hutton Institute, Information and Computational Sciences, Aberdeen, United Kingdom*

Background: Mycobacterium avium subspecies paratuberculosis (Map) causes Johne's disease in cattle, sheep and other ruminants, causing considerable economic losses. It is characterised by the thickening of the gut wall, resulting in diarrhoea, progressive weight loss, and death. While the faecal-oral route is the primary route of disease transmission, studies suggest that other environmental factors are also important for disease prevalence. We aimed to characterise Map prevalence across the country and use this to develop a risk-based sampling strategy to detect Map on working farms.

Objectives: 1) test various soil types for Map presence, 2) develop a model that predicts the probability of Map presence, 3) test this model on working farms

Methods: Soils were collected on a 20km grid across the country. The prevalence of mycobacteria and Map were assessed by QPCR of the IS900 and F57 loci, respectively. These results, and associated soil chemistry and environmental data were used to develop a risk-based model, which predicts probabilities of Map presence. This was tested on several beef and dairy farms, where areas were selected and tested for Map.

Results: The spatial distribution of Map prevalence was determined across the country. Map presence was associated with altitude, iron, carbon, pH and herd size. The majority of the areas on the working farms identified by the model as 'high risk' were indeed positive for Map (69%). This novel risk-based tool can be used by farmers to idenifiy environmental reservoirs of Map, which can be targeted for control methods.

PW090 Fluctuations of the composition of the microbial community of oral biofilms upon dietary changes

Annette Carola Anderson[1], Lamprini Karygianni[2], Johan Woelber[1], Markus Altenburger[1], Elmar Hellwig[1], Ali Al-Ahmad[1]

[1]Albert-Ludwigs-University Freiburg, Department of Operative Dentistry and Periodontology, Faculty of Medicine, Freiburg, Germany
[2]University of Zürich, Clinic of Preventive Dentistry, Periodontology and Cariology, Center for Dental Medicine, Zürich, Switzerland

Background: Caries development is associated with increased numbers of acidogenic species in dental biofilm due to frequent simple carbohydrate consumption. Direct influences of dietary changes on these microbial communities remain unclear.

Objectives: The influence of three dietary phases (high in carbohydrate, dairy and fibers respectively) on dental biofilm compositions compared to the normal diet was studied with culture and molecular cloning technique.

Methods: Eleven individuals wore splint systems containing bovine enamel slabs for 3x7 days with intervals while keeping their regular diet (phases I and V) to obtain dental biofilm. Dietary changes, 3 months duration each followed (5x 2g rock candy daily in phase II, 250g dairy products in phase III, 500g vegetable puree in phase IV). Biofilm samples were collected in each phase and the diet of the individuals was monitored. The microbial community of the dental biofilm was analyzed with culture method and 16S rDNA cloning technique.

Results: Oral biofilm communities were dominated by oral streptococci. These increased in phase II and decreased again significantly in phase V. Concentrations of Actinomyces spp. and Rothia spp. significantly decreased in phase V and Neisseria spp. decreased in phase III and V. Culture and cloning technique revealed similar fluctuations. In conclusion, a dietary change with frequent simple carbohydrate consumption increased oral streptococci, among them acidogenic and aciduric species. Following dietary phases high in dairy and high in fibers, streptococci and Actinomyces spp. decreased again. The observed fluctuations of the microbial community support the extended ecological plaque hypothesis.

PW091 antimicrobial activity of surfactants expressed by rhizosphere associated bacteria on Escherichia coli and Staphylococcus aureus

Kamaluddeen Kabir[1], Bashir Abdulqadir[1]

[1]*Umaru Musa Yar'adua University Katsina, Microbiology, Katsina, Nigeria*

Background: Biosurfactants are surface-active compounds that has both hydrophobic and hydrophilic moiety. They reduce the surface tension of water and are increasingly used in Biotechnology including use as biocontrol, food emulsifiers among others. Several studies have shown that, surfactant-expressing strains are rapidly isolated from both soil and water environments.

Objectives: To isolate surfactants expressing bacteria and to screen the surfactant produced for antmicrobial activity with a long-term goal of identifying novel surfactant that could be use as drug or disinfectant.

Methods: A large collection of 216 rhizosphere-associated bacterial strains were isolated from Umaru Musa Yar'adua Botanic Garden soil. The isolates were screened for surfactant expression using the drop-collapse assays and blood haemolysis technique. Moreover, strains found to be positive where further characterized using different metabolic and growth-based assays including productions of enzymes such as gelatinase, protease and catalase to establish diversity amongst the isolates. Similarly, Antimicrobial properties against *E. coli* and *S. aureus* were tested using agar wall diffusion method.

Results: A total 28 bacterial strains were found to be positive for surfactant-expression. Similarly, Hierarchical cluster analysis of the metabolic and growth-based assays data revealed a significant diversity amongst the isolates. Moreover, 15 isolates where selected and further studied their antimicrobial properties against *Escherichia coli* and *Staphylococcus aureus*. The result shows that only 3 semi-purified compounds possess 4.0mm, 4.5mm and 6.8mm zone of inhibition against *E. coli* and none of the semi-purified material has activity against *S. aureus*. Further research is needed to evaluate these three compounds and their potentialities.

PW092 ureolytic fungi as geoactive agents: environment and bioremediation

Qianwei Li[1]

[1]*College of Chemical Engineering and Envrionment, Environmental Science and Engineering, Beijing, China*

Background: Urease-positive fungi play important roles in soil or aqueous solution for toxic metal or radionuclide bioremediation, with the ability to immobilize toxic metals or radionuclides efficiently by precipitation or coprecipitation, irrespective of metal valence state and toxicity and the redox potential.

Objectives: The overall aim of this project is to examine fungal biomineralization of metal carbonates to provide further understanding of the mechanisms involved and its environmental significance and possible applications for the synthesis of novel biomaterials and in metal bioremediation or biorecovery.

Methods: The ureolytic fungi were incubated in urea and metal ion-containing (Ca^{2+} and/or Sr^{2+}) medium at 25^{o}C for 12 days, and the resulted biominerals were analyised by scanning electron microscopy (SEM), energy-dispensive X-ray analysis and X-ray diffraction (XRD).

Results: After incubation, calcite ($CaCO_3$), strontianite ($SrCO_3$), vaterite in different forms ($CaCO_3$, $(CaxSr_{1-x})CO_3$) and a novel mineral olekminskite ($Sr(Sr,Ca)(CO_3)_2$) were precipitated, and this is the first discovery of the biomineralization of olekminskite and coprecipitation of Sr into vaterite mediated by fungi. In addition, fungal "footprints" were observed on mineral surfaces which indicated that fungal hyphae might provide nucleation sites for mineral formation and thus stabilize the carbonate minerals precipitated. These findings suggest that ureolytic fungi could play an important role in the environmental fate of Sr, and have potential for bioremediation or biorecovery of Sr or other metals and radionuclides that form insoluble carbonates.

PW093 Soil properties and vegetation coverage affect bacterial communities in Western Greenland

Fabiana Canini[1;2], Laura Zucconi[1], Laura Selbmann[1;3], Silvano Onofri[1], József Geml[2]

[1]*University of Tuscia, Department of Ecological and Biological Sciences, Viterbo, Italy*
[2]*Naturalis Biodiversity Center, Biodiversity Dynamics, Leiden, Netherlands*
[3]*Italian National Antarctic Museum (MNA), Mycological Section, Genova, Italy*

Background: Climate changes, that are particularly affecting the Arctic, are resulting in a dramatic expansion of shrubs at the expenses of other land cover types. The potential effect of these phenomena on the structure and functioning of soil bacterial communities are still difficult to forecast, because despite the direct effect of vegetation coverage in shaping the associated microbial communities and the role of bacteria in supporting the colonization of plants, very few studies have addressed the possible effects of changes in vegetation composition on the associated communities in arctic environments.

Objectives: Our main aim was to understand how diversity and functional composition of bacterial communities are related to microhabitats representing a gradient of increasingly complex vegetation: bare ground, biological soil crust, and vascular vegetation. Obtained data could provide better understanding of how environmental factors (edaphic variables and vegetation) shape bacterial communities and their possible response to climate change.

Methods: We used soil DNA metabarcoding to characterize bacterial richness and community composition in the above microhabitat types in Western Greenlandand to infer functionality based on predicted enzyme production profiles. Furthermore, we explored correlations between bacterial community composition and edaphic parameters and shrub community composition.

Results: Richness and community turnover of bacterial communities at different taxonomic levels differed among microhabitats and correlated with edaphic parameters, as did their inferred metabolic profiles. Additionally, in vascular vegetation plots, shrubs composition had a strong influence on bacterial community composition.

PW094 Metagenomic analysis of bacterial community richness and diversity in Belgrade Sava River alluvial aquifers

Ivica Dimkić[1], Vesna Obradović[2], Branko Jovčić[3;4], Goran Vukotić[3], Milan Kojić[4], Milan Dimkić[2]

[1]*University of Belgrade Faculty of Biology, Microbiology, Belgrade, Serbia*
[2]*Institute for the Development of Water Resources "Jaroslav Černi"*
[3]*University of Belgrade Faculty of Biology, Biochemistry and Molecular Biology*
[4]*Institute of Molecular Genetics and Genetic Engineering (IMGGE), University of Belgrade*

Background: River aquifers are populated by a vast diversity of microorganisms and groundwater quality can be influenced by microbiological processes, while biological activity of bacteria can serve as an ecosystem status indicator and biological generated corrosion and fouling may have a direct impact on aquifer ageing.

Objectives: This study aimed to: characterize and evaluate the spatial dynamics and changes of the total bacterial communities; determine whether these changes could be linked to variations of environmental conditions; and sensitivity of various sources of pollutants and undesirable bacteria.

Methods: In this study, eleven Belgrade Sava river alluvial aquifers and one piezometer were investigated, using 16S rDNA 454 Life Sciences GSFLX Titanium sequencing.

Results: Generally, more than 26 bacterial phyla with 395 of different bacterial taxa were revealed. Shannon, Simpson and Chao1 indices confirmed that the bacterial diversity was high but significantly varied between aquifers. The richest biodiversity in bacterial taxa was noted in aquifers **RB-5m**, **RB-15-1**, **RB-38** and **RB-48**, with the frequent presence of sulfur compounds oxidizers and nitrate reducers. Principal coordinate analysis showed that aquifers **RB-13** and **RB-16** were phylogenetically the most distant among all aquifers, with more than 47% of variability, the lowest number of bacterial taxa and the highest percent of facultative anaerobes. Site series investigation along the alluvial aquifers also highlighted habitat specialization for some major members of the community (genus *Thiobacillus*). It can be assumed that the low content of oxygen and organic carbon favours species that could use iron, sulphides and/or nitrates compounds in their metabolism.

PW095 Detection and antibiotic susceptibility profile of Escherichia coli isolated from the final effluent of two wastewater treatment Plants in the Eastern Cape Province, South Africa

Olayinka Osuolale[1], Anthony Okoh[2]

[1]*Elizade University, Biological Sciences, Ilara-Mokin, Nigeria*
[2]*University of Fort Hare, Alice Campus, Alice, South Africa*

Background: Wastewater effluent is an important reservoir for pathogenic and antibiotic resistance *Escherichia coli* which can present significant acute danger if released into surface water without adequate treatment.

Objectives: We examine treated effluents whether pathogenic *E. coli* strains that cause infections are present and to determine their antibiotic profile.

Methods: Culture based approach using *E. coli* differential chromogenic agar for isolation by membrane filtration method. *E. coli* strains as presumptive isolates were purified and confirmed using PCR. The confirmed isolated were also genotyped to identify the species present. The antibiotic profiling of the confirmed isolates were achieved using the CLSI recommended first line antibiotics for *E. coli*.

Results: 476 confirmed isolates from two treatment Plants were characterized for the presence of various *E. coli* pathotypes. A total of 8 pathotypes were screened and only four were confirmed. Uropathogenic *E. coli* (UPEC) was about 5.7% followed by Enteroaggregative *E. coli* (EAEC) at 2.3%, Neonatal meningitis *E. coli* (NMEC) at 1.1% and Enteropathogenic *E. coli* (EPEC) at 0.6%. Antibiotic susceptibility patterns of *E. coli* pathotypes such as UPEC showed low resistance to antibiotics like meropenem (100%), cefotaxime (100%) and gentamicin (88.9%). The pathotype also showed high degrees of resistance to tetracycline (74.1%), ampicillin (74.1%) and cephalothin (66.7%). Other *E. coli* pathotypes, EAEC, NMEC and EPEC, showed high sensitivity (100%) to meropenem, gentamicin and cefotaxime, and varying degree of resistances to ampicillin, tetracycline and cephalothin. This study reveals inadequacy of the plants studied to produce effluents of acceptable quality.

PW096 Fouling microbial communities on plastics compared with wood and steel: are they substrate- or location-specific?

Raeid Abed[1], Maryam Al Khaburi[1], Thirumahal Muthukrishnan[1]

[1]*Muscat, Biology, Muscat, Oman*

Background: Although marine biofouling has been widely studied on different substrates, information on biofouling on plastics in the Arabian Gulf is limited

Objectives: To investigate substrate- and location-specific effects on microbial communities developing on polyethylene terephthalate (PET) and polyethylene (PE) in comparison with those on steel and wood, at two locations in the Sea of Oman

Methods: Illumina MiSeq sequencing of 16S rRNA genes, SEM and FTIR

Results: PET had the highest bacterial abundance at both locations, whereas chlorophyll *a* concentrations did not vary between substrates. MiSeq 16S rRNA sequencing revealed comparable operational taxonomic units (OTUs) richness on all substrates at one location but lower numbers on PET and PE at the other location. Non-metric multidimensional scaling (NMDS) showed distinct clusters of the bacterial communities based on substrate (ANOSIM, $R=0.45\text{-}0.97$, $p<0.03$) and location (ANOSIM, $R=0.56$, $p<0.0001$). The bacterial genera *Microcystis* and *Hydrogenophaga* and the diatoms *Licmophora* and *Mastogloia* were specifically detected on plastics. *Desulfovibrio* and *Pseudomonas* spp. exhibited their highest abundance on steel and *Corynebacterium* spp. on wood. Scanning electron microscopy (SEM) revealed fissure formation on PET and PE, indicating physical degradation. The presence of free radicals on PET and carbonyl bonds (C=O) on PE, as revealed by fourier-transform infrared (FTIR) spectroscopy, indicated abiotic degradation while hydroxyl groups and spectral peaks for proteins and polysaccharides on PE indicated biotic degradation. We conclude that fouling microbial communities are not only substrate-specific but also location-specific and microbes developing on plastics could potentially contribute to their degradation in the marine environment.

PW097 Tracking of the activity of individual bacteria in temperate forest soils shows guild-specific responses to seasonality

Petr Baldrian[1], Tomáš Větrovský[1], Salvador Lladó[1]

[1]*Institute of Microbiology of the CAS, Laboratory of Environmental Microbiology, Praha 4, Czech Republic*

Background: Bacteria belong to the key players in the carbon cycling in forest soils and thus affect the global C balance. Our understanding of bacterial contribution to ecosystem processes relies on the analysis of whole communities.

Objectives: To track the transcriptional activity of individual bacterial species was analysed in the topsoil of a temperate coniferous forest and to assign them to functional guilds.

Methods: Here we combined strain isolation, genome sequencing, metagenomics and metatranscriptomics to describe bacterial activity and contribution to soil processes.

Results: Our results show that bacterial activity is shaped by their habitat and transcription profiles in litter and soil thus differ. Importantly, transcript pools also significant differ between summer when primary producers are active and winter. We show that *Acidobacteria* and *Bacteroidetes* with high metabolic capacity for polysaccharide decomposition in their genomes actively transcribe corresponding genes *in situ*. The rate of the transcription of ribosomal proteins, a proxy of growth, seasonally differs between these “decomposer” taxa and other “opportunistic” bacteria. While the former grow at similar rates in summer and winter, the latter grow much faster in summer when labile C, delivered by plant roots, is available. This paper demonstrates differences in activity of bacterial guilds as well as the importance of environmental drivers for the activity of individual bacteria.

PW098 Efficient Biovolatilization of Tellurium from Water Phase by Pseudomonas stutzeri NT-I

Masashi Kuroda[1], Haruhiko Hatanaka[1], Yuto Suegami[2], Daisuke Inoue[1], Michihiko Ike[1]

[1]*Osaka University, Graduate School of Engineering, Osaka, Japan*
[2]*Osaka University, School of Engineering, Osaka, Japan*

Background: Tellurium (Te) is known as a minor metal in high demand for semiconductor production, and also as a potential pollutant of water environment that may emerge along with the increasing industrial use of Te. An efficient selenium voltilizing bacterium, *Pseudomonas stutzeri* NT-I, may also volatilize Te, which enables development of bioprocess that simaltaneously removes and recovers Te from water phase.

Objectives: This study characterized the Te-volatilizing ability of *P. stutzeri* NT-I.

Methods: Strain NT-I was aerobically cultivated in triptic soy broth medium containing 0.1 mM Te(IV) in jar fermenter. Te concentrations in liquid and solid phases were periodically quantified with inductively coupled plasma atomic emission spectroscopy, then volatile Te was calculated as the loss from the culture. Chemical structures of volatile Te species were estimated with gas chromatography – mass spectrometry analysis.

Results: After the optimazation of growth conditions, 82% of initial amount of Te (60 µmol) was volatilized in 24 h in a bioreactor operated in batch mode (Fig. 1). It is extremely high eficiency because the best Te volatilizer among the previous reports, *Rhodotorula mucilaginosa* volatilized only 2.8% of Te. Volatile Te species detected in the gas phase were $CH_3Te_2CH_3$, CH_3TeCH_3, and CH_3TeSCH_3, which have similar chemical structures to the volatile Se compounds produced by strain NT-I cultivated in TSB medium containing Se, implying that metabolic pathways of Te and Se volatilizations in strain NT-I are analogous to each other.

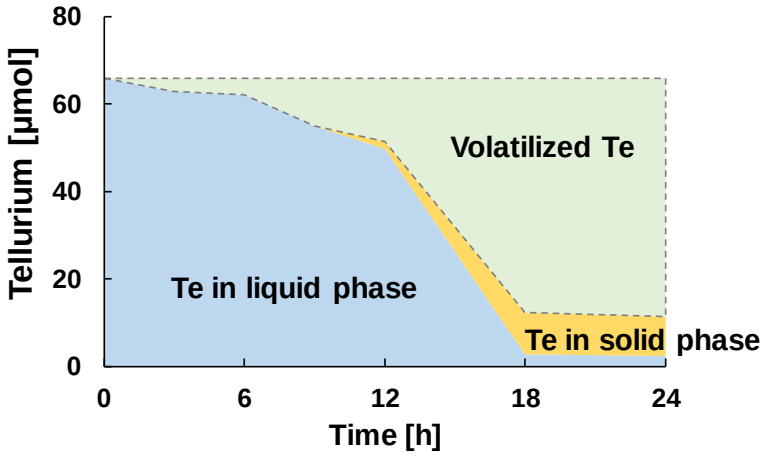


Fig. 1. Transition of Te mass balance during the cultivation of strain NT-I

PW099 The role of temperature on microbial food web structure in the Adriatic Sea

Mladen Solic[1;1], Danijela Santic[1], Stefanija Sestanovic[1], Branka Grbec[2], Frano Matic[2], Zivana Nincevic-Gladan[3], Natalia Bojanic[3], Marin Ordulj[4], Slaven Jozic[1], Ana Vrdoljak[1]

[1]*Institute of Oceanography and Fisheries, Laboratory of Microbiology, Split, Croatia*
[2]*Institute of Oceanography and Fisheries, Laboratory of Physical Oceanography, Split, Croatia*
[3]*Institute of Oceanography and Fisheries, Laboratory of Plankton and Shellfish toxicity, Split, Croatia*
[4]*Univestity of Split, Center for Marine Studies, Split, Croatia*

Background: Temperature is an extremely influencing factor on microbial processes in marine environment. However, these processes have shown different sensitivity to temperature increase that ultimately determines how a microbial community will respond to warming. Along the eastern Adriatic coast sea surface temperature increased by an average of 1.03°C during the period from 1979 to 2015. It is very important to understand the effect of warming on microbial communities, since small temperature increases in surface seawater can greatly modify the microbial role in the global carbon cycle.

Objectives: The main objectives of the study were to check the reproducibility in time of the microbial food web (MFW) structure associated with the change in temperature. We hypothesized that temperature shifts on decadal scale may cause qualitative changes in the MFW structure, which are reproducible in time and similar on the spatial scale regardless of the difference in the trophic status.

Methods: Time series data were analysed using *Self-Organizing Map* (SOM) analysis, which represents an unsupervised clustering method based on a neural network model.

Results: The results show that responses of the microbial food web (MFW) structure to temperature changes are reproducible in time. Furthermore, qualitatively similar changes in the structure of the MFW occurred regardless of the trophic status. The rise in temperature was associated with: (1) the increasing importance of microbial heterotrophic activities (increase bacterial growth and bacterial predator abundance, particularly heterotrophic nanoflagellates) and (2) the increasing importance of autotrophic picoplankton (*Prochlorococcus*, *Synechococcus*, picoeukaryotes) in the MFW.

PW100 Biosorption of precious metal by microorganisms isolated from heavy metal contaminated soil

Chih-Ching Chien[1], Zi-Yi Liao [1]

[1]*Yuan Ze University, Graduate School of Biotechnology and Bioengineering, Taoyuan, Taiwan*

Background: Precious metals such as Gold (Au) and Palladium (Pd) are widely used in industrial and biomedical applications. Recovery of these precious metals has become an area of particular interest. Use of microorganisms for heavy metal contaminated wastewaters treatment and recovery of metals based on biological materials such as microbial biomass has been studied. Biosorption of these rare metals by microorganisms is one of the promising approach for recovery of these metals from industrial wastes.

Objectives: Microorganisms isolated from heavy metal contaminated soil were used for evaluation for their abilities to tolerate Pd and Au. Biosorption capacity of these precious metals by these isolates were also examined.

Methods: Bacteria were isolated from contaminated soil using Luria Bertani medium supplemented with Pd or Au. Cell growth was measured as optical density at 600 nm (OD_{600}). Concentrations of Pd and Au remaining in the solution were determined by inductively coupled plasma optical emission spectrometry (ICP-OES).

Results: Two strains of bacteria able to tolerate high concentrations of Pd were isolated and identified as *Ochrobactrum* sp. strain 71 and *Microbacterium* sp. strain 73, respectively. Both strains could grow in the medium containing Pd up to 360 ppm and showed the ability to adsorb 20% of Pd in growth medium. In contrast, biosorption of Au by both bacteria was not apparent when grown in medium containing Au. Extracellular polysaccharides of the bacteria were purified and evaluated for the ability to specifically adsorb Pd.

PW101 Supplementing a biotrickling filter with a quorum-sensing molecule improves the removal of waste gas containing toluene emitted from tape manufacturing plants

Chih-Yu Chen[1], Teh-Hua Tsai[2], Pei-Yu Chen[3], Ying-Chien Chung[3]

[1]Hsing Wu University, Department of Tourism and Leisure, Taiwan
[2]National Taipei University of Technology, Department of Chemical Engineering and Biotechnology, Taiwan
[3]China University of Science and Technology, Department of Biological Science and Technology, Taiwan

Background: Toluene is toxic and is used as industrial solvents. Thus, removing toluene from air streams is necessary. However, a long gas retention time (GRT) and poor efficiency restrict the use of the current biological treatment of toluene.

Objectives: To solve the problem of removing high toluene concentrations at a short GRT, a quorum-sensing molecule [N-(3-oxododecanoyl)-L-homoserine lactone] (OHL) was added to the bioreactor.

Methods: A biotrickling filter (BF) was used to treat synthetic waste gas and actual waste gas containing toluene emitted from tape manufacturing plants. Extensive tests were conducted to determine removal efficiency, removal characteristics, and the bacterial community of the biotrickling filter.

Results: The addition of 20 μM OHL to BF caused a 33% increase in toluene removal, and an average of 99.7% toluene removal was achieved at a short GRT (30 s) when actual waste gas containing toluene (inlet concentration: 502–1350 ppmv) was treated during 98 days of operation. The maximum removal capacity for toluene was 337.9 g/m^3-h. This BF also exhibited satisfactory adaptability to shock loading and shutdown periods. The predominant bacteria in the system were Burkholderiaceae, Comamonadaceae, Pseudomonadaceae, Mycobacteriaceae, Bradyrhizobiaceae, Bacillaceae, Hyphomicrobiaceae, and Rhizobiaceae, as determined through next-generation sequencing; all these bacteria were related to toluene or aromatic degradation. In addition, the low moisture content (~40%) for microbial survival and the low pressure drop (~32.7 mm-H_2O/m) for system operation demonstrated that the system was energy efficient and economical. Thus, the BF with added OHL is a feasible system for achieving superior toluene removal.

PW102 Involvement of PvdRT-OpmQ and MdtABC-OpmB transporters in pyoverdine secretion in Pseudomonas putida KT2440

Tania Henriquez[1], Nicola Stein[1], Heinrich Jung[1]

[1]*Ludwig-Maximilians Universität München, Biozentrum, Department Biologie I, Ber. Mikrobiologie, Martinsried, Germany*

Background: Fluorescent pseudomonads produce a siderophore called pyoverdine, which is secreted in order to capture iron when it becomes scarce. The molecular basis of pyoverdine secretion is only partially understood.

Objectives: We aimed to explore the impact of the putative PvdRT-OpmQ and MdtABC-OpmB efflux systems in pyoverdine secretion in the soil bacterium *Pseudomonas putida* KT2440.

Methods: Genes of the respective systems were deleted and the resulting strains were phenotypically characterized including colony morphology assays and analyses of growth under iron-rich and iron-limited conditions. Also, we quantified the secretion and cellular accumulation of pyoverdine by fluorescence spectroscopy and microscopy. Furthermore, we assessed the effect of the overexpression of the transporters in pyoverdine secretion.

Results: The single deletion of *pvdRT-opmQ* reduced secretion of pyoverdine into the medium and decreased growth under iron limitation, while the deletion of *mdtABC-opmB* did not affect growth. However, deletion of both efflux systems significantly reduced growth and pyoverdine secretion under iron limitation, and caused an accumulation of pyoverdine in the periplasm. Overexpression of *pvdRT-opmQ* led to the opposite effect. These results provide first evidence for an involvement of the multidrug efflux system MdtABC-OpmB in pyoverdine secretion. In addition, the PvdRT-OpmQ system was shown to contribute to pyoverdine secretion in *P. putida* KT2440, extending previous investigations on its role in *Pseudomonas* species. Since the double deletion mutant still secrets pyoverdine, the involvement of other efflux systems in pyoverdine secretion is currently explored.

Henríquez, T., Stein, N. V. and Jung, H. (2018) *Environ Microbiol Rep.* doi:10.1111/1758-2229.12708

PW103 haracterisation of microbial isolates of soak water of "ofada" rice

Oluwafunmilayo Adeniran[1;2], olusegun atanda[3], adewale obadina[4]

[1]*Federal University of Agriculture, Food science and Technology, Abeokuta, Nigeria*
[2]*federal university of agricuture, food science and technology, abeokuta, Nigeria*
[3]*mcpherson university, Academic planning unit, abeokuta, Nigeria*
[4]*federal university of agriculture, food science and technology, Abeokuta, Nigeria*

Background: "Ofada" (*Oriza glaberrima*) is a popular local rice cultivar in Nigeria that is relished for its flavour and aroma which develops during the traditional processing method.

Objectives: This study is aimed at investigating the microbial dynamics of the soak-water of "ofada" rice using phenotypic and genotypic methods.

Methods: The microbial community of the soak-water was investigated at 24h intervals for 120h by the traditional cultural and molecular methods

Results: Three major clusters of bacteria namely; lactic acid bacteria, *Bacillus* spp and the enterobacteriacaea while cluster of fungi (yeast, Aspergillus and Mucor) were isolated from the soak-water by the traditional cultural method. The Sequencing of their 16S rRNA genes revealed a cocktail of bacteria with *Enterococcus feacium* being the predominant bacteria while sequencing of the yeast's ITS 5 genes revealed *Pichia kudriavezii* as the predominant yeast. The neighbour joining Phylogenetic analysis of the sequenced bacteria showed that *Enterococcus feacium, Enterococcus lactis and Enterocccus duran* were closely related.

PW104 Diet and developmental stage affect the bacterial community of the food-waste reducing insect Hermetia illucens

Elena Crotti[1], Matteo Callegari[2], Ramona Marasco[2], Marco Fusi[2], Costanza Jucker[1], Francesca Mapelli[1], Sara Borin[1], Sara Savoldelli[1], Daniele Daffonchio[2]

[1]Università degli Studi di Milano, Dipartimento di Scienze per gli Alimenti, la Nutrizione e l'Ambiente, Milan, Italy
[2]King Abdullah University of Science and Technology, Biological and Environmental Sciences and Engineering Division, Thuwal, Saudi Arabia

Background: The microbial community inhabiting the insect gut is primarily involved in many aspects of the host biology and physiology e.g. nutrition and immunity. Host-intrinsic factors, such as developmental stage and genetics, as well as insect diet affect the assembly of the associated microbial community.

Objectives: Our aim was to investigate the influence of three different diets (i.e. standard, fruit waste and vegetable waste) on the growth performance and gut bacterial community structure of the black soldier fly (BSF) *Hermetia illucens* (Diptera: Stratiomyidae) considering larval, pupal and adult developmental stages. BSF is an efficient bioconverter of organic waste and an interesting alternative protein source as livestock feed in the circular economy framework.

Methods: Diet effect was evaluated on i) insect growth, development and survival, ii) gut physicochemical conditions, and iii) gut bacterial community structure and networking. The gut bacterial community was analyzed by 16S rRNA high-throughput sequencing.

Results: Diet significantly influenced BSF growth performance, as well as the physicochemical conditions (oxygen partial pressure, redox potential and pH) occurring in the insect gut compartments. Significant differences were found among the bacterial communities both considering the developmental stages and the diet sources. Diet-specific bacterial components and key players involved in the host sustenance were revealed. In conclusion, our results indicate that diet and developmental stage are important to shape BSF bacterial community and suggest that diet-driven microbial clades could contribute to the host growth and development.

PW105 Effects of repeated wet-dry events on microbial community dynamics in the context of soil aggregation in four soil types from a climate gradient along the Chilean Coastal Cordillera

Lisa-Marie Moskwa[1], Nadine Bernhard[2], Fabian Horn[1], Carsten W. Mueller[3], Peter Kühn[2], Rómulo Oses Pedraza[4;5], Thomas Scholten[2], Dirk Wagner[1;6]

[1]GFZ German Research Centre for Geosciences, Geomicrobiology, Potsdam, Germany
[2]University of Tübingen, Department of Geosciences, Soil Science and Geomorphology, Tübingen, Germany
[3]Technical University of Munich, Research Department Ecology and Ecosystem Management, Freising, Germany
[4]University of Atacama (CRIDESAT), Copiapó, Chile
[5]University of La Serena (CEAZA), La Serena, Chile
[6]University of Potsdam, Institute of Geosciences, Potsdam, Germany

Background: Terrestrial ecosystems experience regular changes in water availability. These wet-dry cycles have a significant influence on aggregate formation and stabilization processes in soils. The thereby induced changes in the soil moisture regime affects microbial activity and community structure. Even though microbial activity is linked to aggregation processes, specific taxonomic groups which drive the respond and act as potential key players remain largely unclear.

Objectives: The aim of this study was to detect the microbial response patterns to moisture changes and to determine the role of microorganisms in aggregate formation and stabilization processes.

Methods: Aggregate formation was simulated by repeated wet-dry cycles. Four soil types from different climates, ranging from hyper-arid to humid-temperate, were collected along the Chilean Coastal Cordillera. Quantification of bacteria, archaea, fungi, and microbial fingerprinting (DGGE) was used to assess microbial community dynamics. Illumina HiSeq was done to gain a detailed picture of dominant phyla. Shifts in the microbial communities were related to edaphic parameters and aggregate stability.

Results: First results indicate that differences in soil types are more drastically as the moisture effects. A positive response was revealed in the hyper-arid soils, as microorganisms produce dormant stages to deal with the harsh conditions. In the semi-arid and Mediterranean soils, microbial communities remained unaffected and followed parallel responses. In the temperate-humid soils, constant moisture stress resulted in a negative effect, but community diversity increased. Different responses of the soil types are related to a preadaptation of the microbial community to the moisture conditions found in the field.

PW106 2,3,7,8-tetrachlorodibenzo-p-dioxin stimulated by 1,2-dichlorobenzene co-amendment in dioxin-contaminated river sediments

Rachel Dean[1], Cassidy Schneider[1], Haider Almnehlawi[1], Donna Fennell[1]

[1]*Rutgers University, Environmental Sciences, New Brunswick, United States*

Background: Among the chlorinated compounds, polychlorinated dibenzo-*p*-dioxins (dioxins) are at the top of the list in terms of toxicity and persistence in the environment. Due to an upstream industrial company, a local river is historically contaminated with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TeCDD), the most toxic of the dioxin congeners.

Objectives: As organohalide respiring bacteria harbor multiple reductive dehalogenases capable of dechlorinating (and hence detoxifying) several chlorinated compounds, we wanted to test the ability of microorganisms native to the river sediment to dechlorinate dioxins. We also wanted to determine the effects of first enriching for dechlorinating bacteria on trichloroethene and 1,2-dichlorobenzene and the effect of adding either of these organohalides as a co-substrate, to investigate the potential stimulation of a 2,3,7,8-TeCDD dehalogenase.

Methods: We established anaerobic enrichment cultures inoculated with material from 1) original river sediment, 2) river sediment cultures previously enriched on trichloroethene, and 3) river sediment cultures previously enriched on 1,2-dichlorobenzene. We amended microcosms with three dioxin congeners: 2,7-dichlorodibenzo-*p*-dioxin; 1,2,3,4-tetrachlorodibenzo-*p*-dioxin; and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Select sets also received a trichloroethene or 1,2-dichlorobenzene amendment.

Results: We show that after 10 months, dioxins are dechlorinated by bacteria native to the river sediments: We observed dechlorination in all 1,2,3,4-tetrachlorodibenzo-*p*-dioxin-amended microcosm sets; and in one 2,7-dichlorodibenzo-*p*-dioxin- and one 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-amended microcosm set. Sequencing of the inoculum cultures showed the presence of *Dehalogenimonas*, *Dehalococcoides*, *Dehalobacterium* and *Desulfitobacterium*. Further characterization of the bacteria involved in these processes can provide methods for monitoring dechlorination in contaminated sites and potentially lead to new treatment technologies.

PW107 Temporal and spatial distribution of microbial nitrogen-cycling functional genes in two temperate estuaries

Paula Salgado[1;2], Ana Machado[1;2], Adriano A. Bordalo[1;2]

[1]ICBAS - Abel Salazar Biomedical Sciences Institute, University of Porto, Portugal
[2]CIIMAR - Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Portugal

Background: Estuaries are among the most ecologically dynamic and biogeochemically active ecosystems, regulating microbial mediated processes, and playing an important role in global cycles.

Objectives: Our research addresses how environmental factors regulate N-cycling processes in two different estuarine systems, namely the spatial-temporal dynamics of nitrifying and denitrifying communities and an overview of environmental regulation on the non-denitrifying communities through their marker genes distribution.

Methods: Water was collected in two close-by Atlantic temperate estuaries (Ave and Douro, NW Portugal), on a monthly basis during one year. Several environmental indicators were measured, and specific functional genes relative abundance assayed.

Results: In the Ave estuary, higher salinity and temperature correlated with increased relative abundance of *nirK*-type denitrifiers and the non-denitrifying organisms (*nosZ* from clade II), while the denitrifying *nosZ* (from clade I) distribution had higher expression in lower salinity and temperature. Both bacterial and archaeal ammonia oxidizers were positively correlated ($p < 0.01$) with organic matter, and had a negative association with ammonia. Regarding the Douro estuary, 79.5% of N-cycle functional genes variance was explained by environmental factors. Salinity, dissolved oxygen, and temperature influenced the denitrifying *nosZ*I, non-denitrifying organisms, and the archaeal *amoA* relative abundance. Furthermore, functional genes with a strong correlation revealed a co-occurrence pattern, observed namely by *nirK*-type denitrifiers and the non-denitrifying organisms in the Ave estuary, and the denitrifying *nirS* and the bacterial *amoA* genes in the Douro. Therefore, N-cycle genes had distinct distribution patterns, shaped by the specificities of spatial and temporal dynamics of environmental factors in each estuary.

PW108 Characterization of Biofilms on Different Washing Machine Parts

Pia Praefcke[1], Burga Braun[1], Melanie Bohrisch[1], Ulrich Szewzyk[1]

[1]*Technische Universität Berlin, Institute of Environmental Science & Technology, Berlin, Germany*

Background: Household washing machines are exposed to bacteria of different origins. Next to the colonization of household laundry itself, tap water always carries a specific load of bacteria. This continuous exposure supports biofilm formation on different parts of a washing machine and leads to problems like malodor, biocorrosion and in severe cases to pathogenic risks. To solve these problems, responsible organisms have to be identified.

Objectives: The objective of the study was to determine the biofilm forming microorganisms inhabiting a washing machine after four weeks of conventional usage.

Methods: Biofilms from different parts of the machine, regularly exposed to either laundry, tap water or a combination were sampled. Total DNA directly extracted from the biofilms were used for Illumina MiSeq sequencing.

Results: This study revealed α-Proteobacteria like *Caulobacter* as the most abundant OTUs in all samples, except for the water supply. The washing water and the biofilms on the inside of the outer tub also contained ɣ-Proteobacteria species like *Pseudomonas stutzeri*.

Although many of the bacteria inhabiting the sampling sites are commonly known as waterborne, bacteria genera linked to malodor generation in laundry like *Brevibacterium iodinum* could be found, even in samples that had no connection to the laundry. This indicates *Brevibacterium iodinum* not originating from laundry and skin but from the water.

The composition of bacteria in the water supply was different from all other samples, indicating biofilm formation in washing machines is mostly due to organisms coming from laundry and handling.

PW109 Dynamics of aeromonas species in an estuarine environment

Ana Machado[1;2], Lurdes Lima[1], Eva Amorim[1], Adriano A. Bordalo[1;2]

[1]ICBAS—UP, Institute of Biomedical Sciences Abel Salazar—University of Porto, Porto, Portugal
[2]CIIMAR—UP, Interdisciplinary Centre of Marine and Environmental Research of the University of Porto, Matosinhos, Portugal

Background: *Aeromonas* sp. are ubiquitous in aquatic ecosystems. However, some are recognized as emerging pathogens in humans, associated with gastroenteritis, wound infections, septicemia, and skin diseases. The species *A. hydrophila*, *A. veronii*, *A. caviae*, *A. dhakensis*, and *A. media* are accountable for more than 85% of human cases of aeromonosis. The contact with water is crucial for the transmission of pathogenic strains, including in the fecal-oral route.

Objectives: The aim was to investigate the prevalence of *Aeromonas* species in a temperate estuary, and understand its dynamics in relation with environmental constrains, in order to predict potential risk to human health.

Methods: Multivariate analysis was applied to a 5.5 years time series, to study the link between environmental signatures and *Aeromonas* sp. abundance, estimated by qPCR approach.

Results: *Aeromonas* sp. followed the same temporal trend as total bacteria. Abundances decreased below the detection limit in winter, to peak at the beginning of summer and fall. This trend was similar for all potential pathogenic *Aeromonas* species, with percentages between 40 – 80 % of the total *Aeromonas* abundance. Temperature appears to be the key environmental factor driving the *Aeromonas* species dynamics. Interestingly, fecal indicators, like fecal coliforms and intestinal enterococci, had a negative relationship with the *Aeromonas* species, eventually filling a different ecological niche. The presence of potential pathogenic *Aeromonas* species, especially in the warmer months brings an additional and underlooked health risk to recreational water users.

This work was funded by FEDER (POCI-01-0145-FEDER-031291), and by National Funds from FCT, Portugal.

PW110 A closer look at the microbiome´s dynamics during the start-up phase of biogas fermenters

Katharina Willenbücher[1], Michael Klocke[1], Marius Conrady[2], Dirk Benndorf[3], Andreas Schlüter[4], Patrice Ramm[2]

[1]Leibniz Institute for Agricultural Engineering and Bioeconomy (ATB), Dept. Bioengineering, Potsdam, Germany
[2]Institute of Agricultural and Urban Ecological Projects affiliated to Berlin Humboldt University (IASP), Department of Biogenic Raw Materials, Berlin, Germany
[3]Otto von Guericke University, Max Planck Institute for Dynamics of Complex Technical Systems , Dept. Bioprocess Engineering, Magdeburg, Germany
[4]Bielefeld University, Center for Biotechnology (CeBiTec), Institute for Genome Research and Systems Biology, Bielefeld, Germany

Background: Our study investigates the microbial processes in semi-continuous biogas reactors during anaerobic digestion of lignocellulosic substrates. In our first experiment, we are focusing on the start-up phase. In this initial phase of a biogas process, a functional microbiological community necessary for anaerobic fermentation is formed. Our second experiment will focus on the entire microbial process over the course of one year.

Objectives: The aim is to characterize the structure and functionality of the microbiome as well as its dynamics during the ongoing biogas processes. By a comprehensive analysis of the community at metagenome, metratrascriptome, metaproteome level and a chemical analysis of the start-up phase, it is possible to identify key organisms as well as key characteristics that provide information about the major factors in the biogas process. A special interest lies in the key species responsible for the hydrolysis of lignocellulosic substrates. In addition, the microbial community will be screened for phages.

Methods: An integrated -*omics* approach including subsequent data set analysis using bioinformatic tools is applied. The analysis of metagenome data sets allows determining both the composition of the microbial communities involved in anaerobic digestion as well as their genetic potential for anaerobic biomass degradation and biomethanization. Pre-characterization is performed by monitoring the structure and development of microbial communities using DNA-based TRFLP fingerprinting. The phages are determined by microscopic analyses (transmission electron microscope).

Results: The results of this study will contribute to the understanding of the establishment of functional microbial networks in technical anaerobic digestion processes.

PW111 Novel organohalide-respiring Halodesulfovibrio spp. isolated from estuarine sediments

Jie Liu[1], Emily Choi[1], Lee Kerkhof[2], Max Häggblom[1]

[1]*Rutgers University, Biochemistry and Microbiology, New Brunswick, United States*
[2]*Rutgers University, Marine and Coastal Sciences, New Brunswick, United States*

Background: Organohalide-respiring bacteria, which utilize organohalides as electron acceptors for energy conservation, play an important role in the global halogen cycle. These are found among diverse bacteria phyla and the capacity of organohalide respiration is particularly widely distributed in members of marine *Deltaproteobacteria*.

Objectives: Organohalide compounds are widespread in the environment as a result of both anthropogenic activities and natural production. Our aim was to isolate and characterize organohalide-respiring bacteria from estuarine sediments.

Methods: In this study we isolated two novel organohalide-respiring bacteria after enrichment on bromophenol. The physiology and dehalogenation range of these two isolates were investigated and their genomes were sequenced and analyzed to reveal their metabolic potentials.

Results: Two anaerobic bacteria with dehalogenating ability, strain AK and strain HS, were isolated from estuarine sediments. These bacterial strains are sulfate reducing *Deltaproteobacteria* that can dehalogenate a variety of brominated compounds. Their growth is supported by lactate and 2,6-dibromophenol, with respiratory reductive dehalogenation used for energy generation. Reductive debromination was not inhibited by sulfate. The capability for reductive dechlorination was not observed. Phylogenetic analysis indicates that these two strains represent a novel species, for which we propose the name *Halodesulfovibrio debrominans*. Three reductive dehalogenase genes and the genes encoding for a complete corrinoid biosynthesis are present in their genomes. Transcript analysis shows that expression of one reductive dehalogenase gene in each strain is induced by 2,6-dibromophenol. This study expands our knowledge about the organohalide-respiring potential of *Deltaproteobacteria* and their roles in organohalogen cycling in the marine environment.

PW112 Alcanivorax borkumensis, a key player for low-density polyethylene degradation

Alice Delacuvellerie[1], Valentine Cyriaque[1], Sylvie Gobert[2], Samira Benali[3], ruddy wattiez[1]

[1]*University of Mons, Proteomics and microbiology department, Mons, Belgium*
[2]*University of Mons, Oceanology Department, Liège, Belgium*
[3]*University of Mons, Polymer and Composite Materials Department, Mons, Belgium*

Background: Most plastics are released to the environment in landfills and end up in the sea, inducing large ecological and health impacts. Nevertheless, plastics could constitute a physical substrate and potential carbon source for microorganisms.

Objectives: Highlight new strains of bacteria that could be good candidates for plastic degradation and increase knowledge concerning plastic degradation mechanism.

Methods: In an attempt to select bacteria which potentially degrade different type of plastics (PET, PS, LDPE), an enrichment method was used. Bacterial consortia, sampled from marine plastisphere (Mediterranean, Corsica), were grown with a piece of plastic film as the main carbon source and a low percentage of extra carbon (0.05% yeast extract). After the selection and isolation of *Alcanivorax borkumensis*, its capacity to form biofilm and degrade plastics was monitored by crystal violet and the weight loss methods respectively.

Results: Several Genera containing hydrocarbon-degrading bacteria species were enriched on several plastics and species association were observed including *Alcanivorax borkumensis* specifically selected on LDPE. Results demonstrate clearly that *A. borkumensis* forms large biofilms on the plastic surface and was also able to degrade LDPE. For the first time, we showed its capacity to degrade a solid structure based to alkane such as petroleum-based plastic LDPE. The mechanism of a partial depolymerization of LDPE – a crucial step for its degradation by *Alcanivorax borkumensis* is unknown and will be further investigated.

PW113 Plant growth promoting bacteria: a promising tool in phytodepuration or a risk for antibiotic resistance spread?

Valentina Riva[1], Francesca Mapelli[2], Evdokia Syranidou[3], Nikolas Kalagerakis[4], Redouane Choukr-Allah[5], Sara Borin[2]

[1]University of Milan, Department of Food, Environmental and Nutritional Sciences, Milan, Italy
[1]University of Milan, Department of Food, Environmental and Nutritional Sciences, Milan, Italy
[3]School of Environmental Engineering, Technical University of Crete, Biochemical Engineering and Environmental Biotechnology Laboratory, Department of Environmental Engineering, Chania, Greece
[4]School of Environmental Engineering, Technical University of Crete, Biochemical Engineering and Environmental Biotechnology Laboratory, Department of Environmental Engineering, Chania, Greece
[5]Hassan II Institut Agronomique et Veterinaire, Rabat, Morocco

Background: The urgency to minimize water footprint in agriculture makes water reuse a priority. Constructed wetlands (CWs) represent an economic and sustainable method for wastewater depuration. The microbiome of plants used in phytodepuration can be crucial to improve plant growth under stress conditions and sewage remediation efficiency. Plants could nevertheless constitute a hot spot for the enrichment of antibiotic resistant bacteria (ARB) from wastewaters.

Objectives: This work aims to study the bacterial community of plants commonly used in CW systems and find new powerful microbial inoculants that could ameliorate the functioning of the technology without increasing the risks of ARB spread.

Methods: A bacterial collection obtained from *Phragmites australis* roots has been identified and characterized for plant growth promotion, micropollutant tolerance/degradation, antibiotic resistance. The most promising strains were selected for a CW-microcosm experiments to test their suitability as candidate inoculants for the removal of the azo-dye Reactive Black-5 (RB5) from textile wastewaters.

Results: The root system of *P. australis* plants grown in CW system are naturally associated to a diverse cultivable beneficial microbiome able to promote plant growth and tolerate the presence of micropollutants commonly detected in municipal and industrial wastewaters. Two rhizobacteria applied individually to plants in microcosms and a six-strains consortium enhanced the capacity of *J. acutus* to remove RB5 from the contaminated influents. However, the spread of antibiotic resistance in the collection poses a concern on the possible risks, in terms of environmental safety, of treated wastewater for irrigation purposes.

PW114 Trebon Clade actinobacterium as an effector of bacterial interactions in soil

Daria Rapoport[1;2], Jan Kopecky[2], Marketa Mareckova[2;3]

[1]Faculty of Science, Charles University, Genetics and Microbiology, Prague, Czech Republic
[2]Crop Research Institute, Epidemiology and Ecology of Microorganisms, Prague, Czech Republic
[3]Czech University of Life Sciences Prague, Microbiology, Nutrition and Dietetics, Prague, Czech Republic

Background: Trebon Clade (TC) was described as a dominant actinobacterial group in acid waterlogged soil in Trebon basin (Czech Republic) and was detected in 16S rRNA gene libraries of other acidic soils around the world. Only few isolates were cultivated so far, one – by the authors of the current work. The purpose of the study is to assess the influence of the TC actinobacteria on bacterial community of the acid waterlogged Trebon soil.

Objectives: to assess the ability of the TC actinobacterium to interact with soil bacteria *in silico* and *in vivo.*

Methods: A whole genome of the TC bacterium was sequenced by Illumina MiSeq, assembled with Spades and annotated using RAST and AntiSmash. An innovative interaction experiment was set. Petri dishes were inoculated by pure culture of the TC strain alone and together with soil suspension and incubated at 25°C for up to 14 weeks. DNA samples from „soil suspension“ part of agar plates were extracted, amplified (V4 region of 16S rRNA gene), sequenced by Illumina MiSeq and processed in Mothur.

Results: Biosynthetic gene clusters of secondary metabolites and multiple genes for extracellular enzymes were detected along with significant share of unknown ORFs and a „large“ (>9 Mbp) chromosome of the TC bacterium. Soil bacterial communities significantly differed when growing per se and in the presence of the TC strain, which affected different bacterial groups (including previously uncultured) in a specific manner. Thus, we assumed an important role of the TC group in bacterial interactions in soil.

PW115 Cultivable diversity of methanogenic archaea from Indian hot springs

Kasturi Deore[1;2], Vikram Lanjekar[1;2], Prashant Dhakephalkar[1;2], Sumit Singh Dagar[1;2]

[1]*Agharkar Research Institute, Bioenergy, Pune, India*
[2]*Savitribai Phule Pune University, Pune, India*

Background: Methanogens, best known for their strict anaerobic nature and ability to produce biogenic methane, belong to domain archaea and are present in many oxygen deprived habitats, including hot springs. India is blessed with around 300 hot springs located in different geothermal provinces but, their microbial diversity remains majorly unexplored. The assessment of cultivable methanogen diversity from such environments is crucial because of their potential use in the anaerobic digestion of organic wastes for methane production.

Objectives: The objective of this study was to explore the cultivable diversity of all three groups of methanogens over a broad temperature range from various hot springs in India.

Methods: Sediment and water samples were collected from ten hot springs located in five different geothermal provinces of India. Enrichments were set up for aceticlastic, methylotrophic and hydrogenotrophic methanogens at 40℃, 55℃, 70℃ and 85℃. Following methane production, isolations were performed from positive enrichments using Hungate's roll tube technique.

Results: Nine species of methanogens belonging to *Methanothermobacter, Methanobacterium, Methanoculleus, Methanosarcina*, and *Methanomethylovorans* genera were isolated at 40℃. At 55℃, three species of *Methanothermobacter* namely, *M. thermautotrophicus, M. thermophilus,* and *M. marburgensis* were cultivated whereas, at 70℃ only *M. marburgensis* was obtained. Two of the isolates obtained at 40℃ showed less than 98.7% 16S rRNA gene sequence similarity with *Methanosarcina horonobensis* and *Methanobacterium aarhusense*, indicating their putative novel nature at species level. This study presents the first report of cultivation of methanogens from Indian hot springs and reveals that methanogenic diversity decreases with increase in temperature.

PW116 The contamination fingerprint shapes bacterial community structure and activity in a historically PCB-polluted site: an indication of soil self-depuration potential?

Francesca Mapelli[1], Lorenzo Vergani[1], Elisa Terzaghi[2], Giuseppe Raspa[3], Elisabetta Zanardini[2], Cristiana Morosini[2], Antonio Di Guardo[2], Sara Borin[1]

[1]*University of Milan, Dep. of Food, Environmental and Nutritional Sciences, Milan, Italy*
[2]*University of Insubria, Dep. of Science and High Technology, Como, Italy*
[3]*La Sapienza University, Dep. of Chemical Engineering, Materials, and Environment, Roma, Italy*

Background: Pollutants in historical contaminated sites constitute environmental drivers for the emergence of degrading populations supporting self-depuration potential. Studies aimed at identifying the drivers of microbiome selection are therefore pivotal to develop successful bioremediation strategies.

Objectives: We unravelled the environmental drivers of microbial diversity and activity within the soils of the SIN-Brescia Caffaro (Italy), presenting mixed and uneven contamination particularly by polychlorinated biphenyls (PCBs). Few aerobic PCB degrading strains have been characterized up to now, thus impairing the study of the soil self-depuration potential. Our aim was the evaluation of a correlation between pollutants and microbial fingerprints, an indirect indication of the pollutant role as driver for the enrichment of degrading populations.

Methods: More than 120 soil samples were geostatistically localized and collected from three different former agricultural areas. Bacterial communities structure and abundance, soil microbial hydrolytic activity and aerobic PCB degradation potential were evaluated using molecular methods, and statistical analysis determined the correlation with soil properties.

Results: Statistical analyses showed that significantly different bacterial communities were present a) along the depth profile, b) in the three investigated areas characterized by different pollution levels. Soil properties and pollutant concentrations significantly correlated with the structure and activity of the residing bacterial communities, leading us to hypothesize that the pollution shaped the soil microbiota, possibly driving the selection of degrading populations. The quantification of the biphenyl dioxygenase genes (*bphA*), a molecular marker for aerobic PCB degradation, confirmed that the polluted soils host an intrinsic self-depuration potential, possibly exploitable for future biostimulation interventions.

PW117 Secret collaborations: How plant-associated Archaea interact within the holobiont

Julian Taffner[1], Tomislav Cernava[1], Armin Erlacher[1], Anastasia Bragina[1], Henry Müller[1], Christin Zachow[2], Christine Moissl-Eichinger[3], Gabriele Berg[1]

[1]*Institute of Environmental Biotechnology, Graz University of Technology, Graz, Austria*
[2]*Austrian Centre of Industrial Biotechnology (ACIB GmbH), Graz, Austria*
[3]*Department of Internal Medicine, Medical University of Graz, Graz, Austria*

Background: Plant holobionts harbour a great diversity of microorganisms, such as bacteria and fungi, influencing plant nutrition, resistance to stress and fitness. Furthermore recent studies showed that actually Archaea also shape the microbiome of plants, but their functions and interactions with their hosts remained mostly unclear.

Objectives: To get a broader insight into the community structure, habitat preferences, biogeographical pattern and functions of plant-associated Archaea, we compared 42 different agriculturally used plant-species from the mediterranean area, Austria and Eastern Africa as well as the vegetation of alpine raised bogs from Styria (Austria).

Methods: Therefore we used a combined approach including 16S rRNA amplicon sequencing, whole metagenome shotgun sequencing and fluorescence in situ hybridization confocal laser scanning microscopy (FISH-CLSM).

Results: The highest relative abundances of Archaea were detected in the endosphere of olive trees (Olea europaea L.), with up to 67.3% of total reads, in dwarf shrubs (Vaccinium myrthillus and V. oxycoccus, with 33.0% and 31.7% respectively), and in the rhizosphere of sugar beets (Beta vulgaris L.) with 20.0%. Across all habitats the archaeal community structure was clearly dominated by Euryarchaeota, followed by the less abundant phylum of Crenarchaeota and Thaumarchaeota, except in O. europaea, where Thaumarchaeota were predominant. On plants, we observed signatures for putative adaptation mechanisms of Archaea for their hosts, including those for higher chemotaxis, nutrient cycling like CO2 fixation, stress response, especially against oxidative stress, archaeon stability, and possible plant growth promotion through auxin. These findings reveal a so far unobserved role of Archaea for plant holobionts.

PW118 Understanding how the irrigation of soil with reclaimed wastewater containing contaminants of emergent concern affects the microbial structure: a microcosms approach

Paula Guedes[1;2], Celso Martins[2], Nazaré Couto[1], Joana Silva[1], Eduardo P. Mateus[1], Cristina Silva Pereira[2], Alexandra B. Ribeiro[1]

[1]Faculdade de Ciências e Tecnologia (Universidade Nova de Lisboa), CENSE, DCEA, Caparica, Portugal
[2]ITQB Nova, Oeiras, Portugal

Background: Water resources are under increasing pressure. The use of treated wastewater for irrigation - reclaimed wastewater (RWW) - is an alternative encouraged by the EU. However, the dissemination of contaminants of emergent concern (CECs) in agricultural soils irrigated with RWW has been made apparent. We have previously demonstrated that *in situ* enhanced bioremediation can be achieved by the application of a low-level direct current in the soil - electrokinetic processes. However, and prior to taking advantage of these synergistic effects, there is a need to understand how soil microbiota responds to both RWW and CECs.

Objectives: Understand how RWW containing CECs affects soil microbial community composition and functioning and, consequently, the bioremediation processes

Methods: A clay soil was irrigated with either deionised water or RWW, both of which were spiked or not with a mix of nine CECs. Liquid chromatography was used to analyse the pollutant decay. At each condition under study, the metacommunity composition and functioning was studied using amplicon sequencing and Biolog FF microplates, respectively.

Results: The experiments disclosed the contribution of the indigenous soil microorganisms to the remediation process. The efficiency of the biodegradation was influenced by contaminants physico-chemical characteristics and by the use of RWW. The influence of RWW on biodegradation was more pronounced till the 7th day of exposure, where the soil pH was also higher compared to the corresponding control condition (spiked deionized water). Currently, we are unveiling potential links between the decay of each CEC with the soil microbiota composition and functioning.

PW119 Role of microorganisms in sulfur solubilisation during autotrophic denitrification and denitritation

Anastasiia Kostrytsia[1;2], Stefano Papirio[3], Luigi Frunzo[4], Maria Rosaria Mattei[4], Estefanía Porca[5], Gavin Collins[6], Piet N. L. Lens[7], Giovanni Esposito[8]

[1]*University of Cassino and Southern Lazio, Department of Civil and Mechanical Engineering, Cassino, Italy*
[2]*University of Glasgow, School of Engineering, Glasgow, United Kingdom*
[3]*University of Naples Federico II, Department of Civil, Architectural and Environmental Engineering, Naples, Italy*
[4]*University of Naples Federico II, Department of Mathematics and Applications "Renato Caccioppoli", Naples, Italy*
[5]*National University of Ireland Galway, Microbial Communities Laboratory, Galway, Ireland*
[6]*National University of Ireland Galway, Microbial Communities Laboratory, School of Natural Sciences and Ryan Institute, Galway, Ireland*
[7]*IHE, Institute for Water Education, the Department of Enivronmental Engineering and Water Technology, Delft, Netherlands*
[8]*University of Cassino and Southern Lazio, Department of Civil and Mechanical Engineering, Cassino, Netherlands*

Background: The limited solubility of chemically synthesized elemental sulfur (S^0) remains a major obstacle to full-scale applications in biotechnology of autotrophic denitrification and denitritation. S^0 is taken up by denitrifying microorganisms after its solubilisation and diffusion into the cells. Because of the rather insoluble properties of S^0 particles, a preliminary hydrolysis is required to make S^0 soluble and bioavailable.

Objectives: The objective of this research was to investigate role of microorganisms in S^0 solubilisation during denitrification and denitritation.

Methods: S^0 lentils (particles with an average size between 2 and 4 mm) were used as electron donor for denitrification and denitritation. The microbial community structure was determined at the family-level by sequencing 16S rRNA genes.

Results: *Hydrogenophilaceae*, with a relative abundance below 0.1% in the inoculum, was by far the largest family present in the batch experiments, both as suspended biomass and biofilm attached onto the S^0 lentils with a relative abundance ranging between 36.7 and 59.9%. The families of *Xanthomonadaceae*, *Comamonadaceae* and *Ignavibacteriaceae* were present both in suspended biomass and lentil biofilms. *Helicobacteraceae* was present only in biofilms, where the relative abundance was up to 37.1% and where these bacteria were most likely associated with S^0 solubilisation, which is the necessary step prior to S^0-driven autotrophic denitrification or denitritation. Additionally, analyses of scanning electron microscopy data indicated significant biomass colonisation of S^0 particles during both autotrophic denitrification and denitritation, demonstrating the potential use of the S^0 lentils as an effective biomass carrier.

PW120 Soil Microbial Communities of Radovesice Brown-Coal Spoil hHap (Czechia) Under Different Reclamation Scenarios

Lenka Sikorová[1;2], Josef Trögl[1], Jan Popelka[1], Pavel Kuráň[1], Iva Roubíková[1], Michal Řehoř[1;3]

[1]*University of Jan Evangelista in Ústí nad Labem (UJEP), Faculty of Environment, Ústí nad Labem, Czech Republic*
[2]*Technical University in Zvolen, Faculty of Ecology and Environmental Sciences, Czech Republic*
[3]*Brown Coal Research Institute, Czech Republic*

Background: Surface brown coal mining requires deposition of large quantities of bulk material, dominantly clay. These soil-heaps require reclamation and soil genesis.

Objectives: Comparison of the differences of soil microbial communities on the Radovesice (Czech republic) spoil dump under different reclamation scenarios (including natural succession site and original localities nearby) of age 8 to 16 years.

Methods: Microbial communities were assessed by PLFA profiling according to ISO 29843-2. Soil enzyme activities were determined by direct incubation of soil with artificial enzyme substrates. Soil was characterized based on agriculture standards.

Results: Natural-succession sites were less developed (in terms of soil nutrients, microbial biomass and plant cover) compared to reclaimed sites. Except of the original site, soil microbial communities were dominated by G- bacteria (upper layer) and Actinobacteria (deeper layers) while the proportion of fungal PLFA was lower. The results confirm importance of land reclamations in case the time is the limiting factor.

PW121 Effect of new biocides on Enterococcus hirae biofilm on hospital material surfaces

Paola Di Fermo[1], Silvia Di Lodovico[1], Emanuela Di Campli[1], Simonetta D'Ercole[1], Federica Vasile[1], Elisabetta Ancarani[1], Mara Di Giulio[1], Luigina Cellini[1]

[1]*University "G. d'Annunzio" Chieti-Pescara, Department of Pharmacy, Chieti, Italy*

Background: The microbial contamination in the hospital environment, also related to the capability of bacteria to grow on biofilm mode, is of particular concern because the hospital-acquired infections (HAIs) are responsible for significant morbidity and mortality. *Enterococcus hirae* has been recently described as an emergent nosocomial pathogen in HAIs.

Objectives: This work evaluated the capability of *E. hirae* to form biofilm on different surfaces and the antibiofilm action of two biocides, used in the disinfection of surgical and medical device.

Methods: The biofilm formation of *E. hirae* ATCC 10541 was studied on polystyrene and stainless steel surfaces through the biomass quantification and the cell viability at 20°C and 37°C. The effect of LH IDROXI FAST and LH ENZYCLEAN SPRAY biocides on biomasses, was expressed as percentage of biofilm reduction.

Results: *E. hirae* at 20°C and 37°C produced more biofilm on the stainless steel in respect to the polystyrene surface. The amount of viable cells was greater at 20°C than with 37°C on the two analyzed surfaces. Biocides revealed a good antibiofilm activity. LH ENZYCLEAN SPRAY is most effective on polystyrene and stainless steel at 37°C with a biofilm reduction of 85.72% and 86.37%, respectively.
E. hirae is a moderate biofilm producer depending on surface material and temperature and the analyzed biocides express a remarkable antibiofilm action.
The capability of *E. hirae* to form biofilm can be associated to its increasing incidence in HAIs and the adoption of suitable disinfectants is strongly recommended.

PW122 Development of biopreparation for plant protection under low temperature conditions using Antarctic psychrotolerant bacterial strains

Andrei Tryhubovich[1], Vladislav Miamin[1], Alina Arashkova[1], Emily Kolomiets[1]

[1]*Institute of Microbiology, National Academy of Sciences, Belarus, Biotechnology of Biological Control Agents, Minsk, Belarus*

Background: Extreme conditions of Antarctic (low temperatures, exposure to hyperdoses of solar UV radiation) develop resistance of polar microorganisms to permanent stress factors. The indigenous microbial specter are distinguished by enhanced generation of bioactive agents determining their protective functions, including antibiotic compounds, which makes isolation, selection and characterization of novel strains an extremely relevant and promising research mission.

Objectives: Our objective was to isolate psychrotolerant microorganisms colonizing materials of natural and anthropogenic origin from East Antarctica, alalyze their resistance to adverse enviromental conditions, study their biological activity and select strains with increased production of antimicrobial compounds.

Methods: For investigation were used different microbiological, molecular-genetical and biochemical methods.

Results: Over 150 bacterial cultures from samples of various materials brought from East Antarctica (region around Belarusian field station Mount Vechernyaya). For 98 isolates, ability to develop actively at temperatures close to 0°C was shown. Psychrotolerant Antarctic bacteria were characterized by increased resistance to UV radiation, osmotic stress, toxic effect of heavy metal ions. Among studied strains highly antagonistic cultures with broad spectrum of activity against phytopatogenic microorganisms were screened. Using MALDI-TOF mass spectrometry, selected bacterial isolates were identified as *Bacillus mojavensis* species. According to the results significant antimicrobic effect was caused on phytopathogens from genera *Alternaria*, *Botrytis,Pseudomonas, Xanthamonas* in wide temperature range, including 5-15°C. Test with cultural liquid sample (2% concentration) demonstrated 81% decline of infection rate in seedlings. We assume that isolated strains of *B. mojavensis* are promising constituents for biopreparation formulated for protection vegetable crops from pathogens at low temperature.

PW123 Biotechnology for art conservation: Microbiological characterization of public outdoor sculptures

Nádia Silva[1], Manuela Pintado[1], Patrícia R. Moreira[1;2;3]

[1]Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Porto, Portugal
[2]Universidade Católica Portuguesa, CITAR - Centro de Investigação em Ciência e Tecnologia das Artes, Porto, Portugal
[3]Universidade Católica Portuguesa, Escola das Artes, Porto, Portugal

Background: Microorganisms growing on art objects and cultural heritage can have a significant damaging impact on its structure and aesthetical characteristics, contributing to its deterioration and loss of value. Particularly, public outdoor sculptures are very susceptible to biodeterioration due to exposure to environmental elements, which accelerate the favorable conditions for the colonization of degrading microorganisms. Therefore, an assessment of the microbial contamination of outdoor sculptures is fundamental to understand the biodegradation of these cultural objects and to develop innovative solutions for its preventive conservation.

Objectives: the aim of this work was to characterize the surface microbiome of selected outdoor stone sculptures using non-invasive sampling techniques. The identification of the main groups of bacteria and fungi thriving on the surface of the sculptures will allow us to design appropriate antimicrobial coatings for protection and preservation of stone sculptures against microbial degradation.

Methods: samples from stone sculptures were collected using swabs and a protocol was used to eliminate debris and prepare the samples for further analysis. Flow cytometry was used for quantification of microorganisms and determination of general cell viability. Identification of culturable and unculturable microorganisms was done by sequencing the V3-V4 and ITS2 regions of bacterial and fungal populations.

Results: the results allowed us to identify and quantify the main groups of microorganisms present on the surface of the sculptures, as well as to identify the regions within each sculpture with higher microbial colonization. This microbial characterization will help us design suitable antimicrobial coatings for preventive conservation of stone sculptures.

PW124 Genome mining and transcriptional analysis of bacteriocins, polyketides, and nonribosomal peptides in paenibacilli from Krubera-Voronja Cave

Jolanta Lebedeva[1], Gabriele Jukneviciute[1], Rimvydė Čepaitė[1], Nomeda Kuisienė[1]

[1]*Vilnius University, Life Sciences Center, Vilnius, Lithuania*

Background: *Paenibacillus* sp. strains 28ISP30-2 and 23TSA30-6 were isolated from Krubera-Voronja Cave. Our previous results showed that both strains inhibited growth of *Micrococcus luteus*, *Bacillus thuringiensis*, and *Escherichia coli*, while strain 28ISP30-2 was also active against *Pseudomonas*. sp. Preliminary results allowed us to suggest that both primary and secondary metabolites are responsible for high antimicrobial activity of strains 28ISP30-2 and 23TSA30-6.

Objectives: The objective of the current work was to identify genes encoding synthesis of bioactive metabolites as well as to perform transcriptional analysis of these genes in *Paenibacillus* sp. strains 28ISP30-2 and 23TSA30-6.

Methods: Genome sequencing was performed by the GenXPro GmbH (Germany). Bacteriocin mining was performed using BAGEL 3, and polyketide as well as nonribosomal peptide mining was done using antiSMASH 4.0. The SensiFAST SYBR No-ROX Kit (Bioline) was used for qPCR.

Results: In the genome of strain 23TSA30-6, 54 clusters of secondary metabolites were identified including 23 encoding NRPS and 1 transATPKS-NRPS. 11 clusters encoded biosynthesis of known antimicrobial compounds - polymyxin, fusaricidin, tridecaptin, paenilarvin, *etc*. Screening for bacteriocins revealed three classes – class I and class II lanthipeptides, and lassopeptide. Out of 53 clusters of secondary metabolites that were identified in the genome of 28ISP30-2, 16 encoded NRPS, including 4 clusters related to fusaricidin and polymyxin biosynthesis. Bacteriocins lassopeptide, class I lanthipeptide as well as sactipeptide were also identified in the genome of 28ISP30-2. Qualitative and quantitative transcriptional analyses were performed in order to evaluate an impact of nutrients and growth phase on the gene expression.

PW125 Evaluation of decolourisation of reactive dyes by selected yeasts and related enzymatic activity

Marta Mendes[1], Carolina Cassoni[1], Patrícia R. Moreira[1;2], Paula Castro[1], Manuela Pintado[1]

[1] Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Porto, Portugal
[2]Universidade Católica Portuguesa, CITAR - Centro de Investigação em Ciência e Tecnologia das Artes, Escola das Artes, Porto, Portugal

Background: Textile industry generates high amounts of effluents that are discharged in the environment. Dyes are resistant to biodegradation and are potentially harmful to aquatic life. The existent chemical treatments are very costly and generate large quantities of sludge. Alternatives to aid the decolourisation of dyes in textile wastewaters are of major importance.

Objectives: The aim of this work was to access the capacity of selected yeast strains to decolourise specific dyes used in the textile industry and to assess the activity of enzymes related to decolourisation ability.

Methods: Three Everzol dyes were selected for this work. Yeasts LIIIS36B, HOMOGST27AB and HOMOGS20B were cultivated in Normal Decolourisation medium (NDM) supplemented with 100 mg/L of each dye, in 24 well microplates incubated at 25 ºC for 48 h and 100 rpm. Decolourisation was determined spectrophotometrically.
The enzyme activity assays were assessed in intra and extracellular extracts. The extracellular extract corresponded to the supernatant resulting from 1 day of yeast growth in NDM, whereas the intracellular extract was obtained by cellular disruption of the corresponding pellet using glass beads. The enzymatic activities were determined spectrophotometrically and protein and molecular weights were evaluated by Bradford and FPLC, respectively.

Results: LIIIS36B was the most effective and versatile strain in the decolourisation of the three Everzol dyes.
HOMOGS20B absorbed most of the dyes whereas the other two performed true decolourisation. Intracellular extracts presented activity for oxidoreductase and tyrosinase whereas no extracellular enzyme activities were detected.

PW126 Development of Saprolegnia parasitica PCR detection-assay

Dora Pavić[1], Tamara Vujović[1], Anđela Miljanović[1], Ana Bielen[1]

[1]*Faculty of Food Technology and Biotechnology, University of Zagreb, Department for Biochemical Engineering, Zagreb, Croatia*

Background: Some oomycetes from the genus *Saprolegnia* are causing saprolegniosis, a disease of salmonid fish important in aquaculture. *Saprolegnia* species cause significant economic losses in freshwater aquaculture, with *Saprolegnia parasitica* as the most destructive pathogen. Despite its negative effects, no monitoring protocol of *S. parasitica* has been established to date.

Objectives: We aimed to develop PCR-detection assay for *S. parasitica* in order to enable its monitoring in freshwater aquaculture facilities.

Methods: We constructed multiple sequence alignment of 958 internal transcribed spacer (ITS) sequences belonging to various oomycetes. We have identified *S. parasitica* specific regions and created three primer pairs, designated A, B and C. Next, we used gradient PCR and *S. parasitica* (CBS 233.65) genomic DNA as a template to determine optimal annealing temperature for each primer pair.

Results: Primer pairs A and B have amplified unspecific bands that could not be eliminated, but primer pair C amplified a single band of expected size. The sensitivity of the assay with primer pair C was high: lower detection limit was < 1 pg. Also, we have demonstrated the specificity of the assay using a range of *Saprolegnia* species and other, more distantly related Oomycetes.

In conclusion, intense fish farming in large-scale aquaculture can lead to a significant increase in saprolegniosis. *S. parasitica* detection assay developed here will enable early detection of *S. parasitica* in fish farms and therefore help in improving poor farming conditions.

PW127 Beachsafe: vibrio dynamics in bathing water and associated human health risk

Ana Machado[1;2], Eva Amorim[1], Tomás Araújo[1], Adriano A. Bordalo[1;2]

[1]ICBAS—UP, Institute of Biomedical Sciences Abel Salazar—University of Porto, Porto, Portugal
[2]CIIMAR—UP, Interdisciplinary Centre of Marine and Environmental Research of the University of Porto, Matosinhos, Portugal

Background: Vibriosis outbreaks associated with recreational bathing are on the rise in Northern latitudes. *Vibrio cholerae*, *Vibrio parahaemolyticus,* and *Vibrio vulnificus* are human pathogens known to cause diarrheal illnesses and wound infections. Ongoing climate changes are expected to further drive their emergence in coastal waters, and the spread of waterborne infectious diseases globally.

Objectives: The aim was to investigate the prevalence of vibrios in bathing waters, understand its dynamics in relation with environmental constrains, and therefore predict potential risk to human health.

Methods: Distribution of *V. cholerae*, *V. parahaemolyticus* and *V. vulnificus* were investigated in ten beaches over a year, by means of the most probable number-polymerase chain reaction (MPN-PCR). Also, characterization of vibrio isolates, with special attention to genotypes associated with toxicity and antibiotic resistance, was performed.

Results: *Vibrio* sp. were successfully detected in all samples, with abundances ranging 2.23-8.64 Log MPN/L. Although no genes coding toxins were detected, all the potential pathogenic vibrios were found. *V. cholerae* was ubiquous with the highest abundances, whereas *V. parahaemolyticus* and *V. vulnificus* showed lower numbers, peaking in early fall. Temperature, salinity, and particulate matter appear to have a control role. The antibiotic resistance patterns exhibited a wide variability, with isolates from the same *Vibrio* species showing distinct profiles. The results highlighted the potential public health risk associated with pathogenic and antibiotic resistant *Vibrio* species in bathing waters considered safe by the European Union legal criteria.

This work was funded by FEDER (POCI-01-0145-FEDER-031291), and by National Funds from FCT, Portugal.

PW128 A newly described protein prevents encystment in the amoeba Acanthamoeba castellanii

Steven Rolland[1], Luce Mengue[1], Willy Aucher[1], Cyril Noël[1], Yann Héchard[1], Ascel Samba-Louaka[1]

[1]*UMR CNRS 7267, Ecologie et Biologie des Interactions, Equipe Microbiologie de l'eau, Université de Poitiers, Poitiers, France*

Background: Free-living amoebae (FLA) are ubiquitous protozoa that feed on microorganisms such as bacteria. However, some bacteria have evolved to resist amoebal digestion making FLA as vehicle of numerous microorganisms. Due to the strong similarities between amoeba and macrophage phagocytosis, FLA are considered as training ground for pathogenic environmental bacteria. In response to environmental stresses, amoebae can differentiate into a resisting form called "cyst" that may harbour and protect pathogens. This differentiation process named "encystment" involves several proteins but is still largely unknown.

Objectives: The aim of this study is to identify new proteins involved in encystment of the FLA *Acanthamoeba castellanii*.

Results and methods: From a proteomic analysis using *A. castellanii*, we have selected the hypothetical protein ACA1_384820. To study its role, the protein was overexpressed within *A. castellanii*. This overexpression did not change the growth rate but decreased the ATP production. Interestingly, the overexpression inhibited the formation of cysts. The analysis of the ACA1_384820 gene expression, through RT-qPCR, showed a downregulation during encystment. The protein was compared to databases by BLAST. It showed that the protein possess a N-Acetyltransferase domain and the best score were obtained with bacterial proteins, suggesting that its gene could have a prokaryotic origin.

Conclusions: We describe a new protein involved in the encystment of the amoeba, *A. castellanii.* To our knowledge, this is the first study reporting the involvement of a protein with bacterial origin in amoeba encystment. A better characterization of this protein could allow identification of drug target for cyst clearance.

PW129 Mixotrophic Lifestyle of Nitrite Oxidizer Nitrotoga in the Presence of Pyruvate

Kento Ishii[1], Hirotsugu Fujitani[2;3], Yuji Sekiguchi[2], Satoshi Tsuneda[1;3]

[1]*Waseda University, Department of Life Science and Medical Bioscience, Tokyo, Japan*
[2]*National Institute of Advanced Industrial Science and Technology, Ibaraki, Japan*
[3]*Waseda University, Research Organization for Nano and Life Innovation, Tokyo, Japan*

Background: Nitrite-oxidizing bacteria (NOB) can grow on nitrite as an energy source and fix carbon dioxide. It is estimated that approximate 100 moles of nitrite are required to fix one mole of carbon dioxide, and thus growth depending solely on nitrite oxidation is the burden. Indeed, better growth of some NOB members was observed in mixotrophic conditions supplemented with C_2 to C_3 substrates. Among simple organic carbons, pyruvate is an important metabolic intermediate linking glycolysis and TCA cycle. In addition to role of energy and carbon source, pyruvate protects cells from oxidative stress by hydrogen peroxide detoxification. Although culture-based studies provided that NOB can benefit from pyruvate, the effect of pyruvate on metabolism of NOB is still unknown.

Objectives: In this study, we investigated the effect of pyruvate on nitrite oxidation activity and metabolism of a newly isolated nitrite oxidizer.

Methods: A novel nitrite-oxidizing bacterium strain AM1 belonging to genus *Nitrotoga* was isolated, and its genetic features were characterized. Total RNA was extracted from the exponential phase cells, and the expressions of several genes for energy metabolism were measured by RT-qPCR.

Results: Genome annotation suggests that AM1 possesses the metabolic pathways for chemolithoautotrophic and mixotrophic lifestyle. The nitrite oxidation activity was enhanced as the concentration of pyruvate increased. Overall, the mRNA expression levels of targeted genes increased in the presence of pyruvate compared to the control without organic carbons. To gain a comprehensive understanding of adaptation to pyruvate, we perform time-resolved proteomic analysis in progress.

PW130 Microbial carbon utilization in the soil of a temperate forest

Tijana Martinovic[1], Tereza Mašínová[1], Martina Štursová[1], Petr Baldrian[1]

[1]*Institute of Microbiology of the ASCR, v. v. i., Laboratory of Environmental Microbiology, Prague, Czech Republic*

Background: Soil microorganisms mediate several ecosystem processes, however most studies in this regard so far focused on fungi. Studies of bacteria are much more scarce and only in the last years it comes to our attention that bacteria are also capable of degrading complex plant polymers in soil, and their contribution to C cycling may be equally important as that of fungi.

Objectives: This experiment aimed to track the incorporation of carbon derived from different sources in forest soil into the fungal and bacterial biomass using stable isotope probing.

Methods: Following the incubation of microcosms supplied with ^{13}C substrates of varying complexity, substrate respiration rates were determined. Fungal and bacterial taxa incorporating the ^{13}C into their biomass were identified by ^{13}C-DNA separation and amplicon sequencing on Illumina MiSeq.

Results: The highest respiration rate after 1 week of incubation was observed in microcosms with addition of labeled citric acid, followed by glucose, hemicellulose, maize leaf, cellulose and finally chitin. A considerable increase in chitin respiration rate was observed after 3 weeks of incubation. Furthermore, a distinct microbial community was observed in microcosms containing ^{13}C chitin, clearly showing involvement of both fungi and bacteria in its decomposition. The results indicate that numerous microbial taxa are capable of utilization of different sources of C, both plant-derived and fungal biomass. Even though both specialist and generalist taxa were present in the microcosms, the distinct microbial community in ^{13}C chitin suggests specificity and importance of fungal biomass as carbon source in temperate forests.

PW131 Dracaena sanderiana endophytic bacteria interactions: effect of endophyte inoculation on bisphenol A removal

Bongkotrat Suyamud[1], Paitip Thiravetyan[2], Bunyarit Panyapinyopol[1], Duangrat Inthorn[3]

[1]*Faculty of Public Health Mahidol University, Sanitary Engineering , Bangkok, Thailand*
[2] *King Mongkut's University of Technology Thonburi, School of Bioresources and Technology, Bangkok, Thailand*
[3]*Faculty of Public Health Mahidol University, Environmental Health Sciences, Bangkok, Thailand*

Background: Bisphenol A (BPA) is one of the most abundant endocrine-disrupting compounds which is found in the aquatic environment. Biological treatment using plant-bacteria interactions is a promising technique which offers a sustainable approach for decontamination.

Objective: To investigate the effect of plant-bacteria interactions on enhancing BPA removal.

Methods: This research addresses the isolation, screening, characterization and identification of endophytic BPA-degrading bacteria in the rhizo- and endosphere of *D. sanderiana* roots. Plant-bacteria Interactions were assessed regarding BPA removal, phytotoxicity tolerance, phytohormone induction and the degree of bacterial colonization of the plant tissues.

Results: Two plant growth-promoting (PGP) bacterial strains, *Bacillus thuringiensis* and *Pantoea dispersa*, which have high BPA tolerance and can utilize BPA for growth, were used as plant inocula. *P. dispersa*-inoculated plants showed the highest BPA removal efficiency at 92.32 ± 1.23% compared to other inoculated and non-inoculated plants. This was due to a higher population of the endophytic inoculum within the plant tissues which resulted in maintained levels of indole-3-acetic acid (IAA) for the plant's physiological needs and lower levels of reactive oxygen species (ROS). In contrast, *B. thuringiensis*-inoculated plants had a lower BPA removal efficiency. However, individual *B. thuringiensis* possessed a significantly higher BPA removal efficiency compared to *P. dispersa*. This study provides convincing evidence that not all PGP endophytic bacteria-plant interactions could improve the BPA removal efficiency. Different inocula and inoculation times should be investigated before using plant inoculation to enhance phytoremediation.

PW132 Investigation of magnetotactic bacteria using novel approaches

Veronika Koziaeva[1], Lolita Alekseeva[1;2], Marina Sukhacheva[1], Maria Uzun[1;2], Pedro Leão[3], Ekaterina Patutina[1], Tatiana Kolganova[1], Natalia Slobodova[1], Denis Grouzdev[1]

[1]Institute of Bioengineering, Research Center of Biotechnology of the Russian Academy of Sciences, Moscow, Russian Federation
[2]Lomonosov Moscow State University, Moscow, Russian Federation
[3]Instituto de Microbiologia Paulo de Góes, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil

Background: Magnetotactic bacteria (MTB) are found among various taxonomic ranks and have the ability to synthesize intracellular magnetic crystals. Due to the fact that the ability to synthesize magnetosomes is not a taxonomic feature and the presence of closely related species of MTB and non-MTB, it is difficult to identify MTB 16S rRNA gene sequencing. Since separation methods based on magnetotaxis reveal only MTB that are more motile, abundance and diversity of MTB is biased.

Objectives: The aim of this study was to describe the diversity of MTB from Lake Beloe Bordukovskoe (Moscow region) using novel methods.

Methods: For phylogenetic analysis a primer system based on mamK, which is one of the specific genes for all MTB, was designed. A method based on MACS® columns was adopted for the separation of bacteria. Separated cells were used to obtain clonal libraries of 16SrRNA gene and mamK sequences, FISH-TEM (coupled fluorescent in situ hybridization and transmission electron microscopy) analysis and full genome sequencing.

Results: Phylogenetic analysis of mamK revealed 6 OTUs related to MTB. They belonged to the phylum Nitrospirae, the class Alphaproteobacteria, the families Magnetococcaceae and Syntrophaceae. Analysis of 16S rRNA gene sequences showed similar results. To identify the morphology of detected OTUs, FISH-TEM analysis was performed. For the dominant group of phylum Nitrospirae genome was obtained.

The work was carried out using the scientific equipment of Core Research Facility “Bioengineering” with the support of Russian Foundation for Basic Research through grant 18-34-01005.

PW133 Evaluation of novel plant-origin materials for the cleaning/protection of caves belonging to natural and cultural heritage sites

Anthoula Argyri[1], Agapi Doulgeraki[1], Eftychia Varla[1], Chrysoula Tassou[1], Nikos Chorianopoulos[1]

[1]*Institute of Technology of Agricultural Products, Hellenic Agricultural Organization-DEMETER, Lycovrissi, Attica, Greece*

Background: The current study concerns the serious issue of interior alteration observed in many caves due to the development of various microorganisms. This phenomenon has as direct consequence the significant aesthetic and functional degradation of the caves belonging to natural and cultural heritage sites. Literature results have indicated that the organisms normally grown in these cases are non-photosynthetic and/or photosynthetic microflora visible as green or dark color on the cave walls.

Objectives: The main subject of the current study was to address the above problems by replacing chemical bleaches (mainly chlorinated derivatives) with herbal biocides.

Methods: A series of essential oils and hydrosols of plant origin containing bioactive molecules was evaluated. More specifically, 18 essential oils and hydrosols (in laboratory level 2-5 ml) of plants were tested against 35 isolates of bacteria and 24 isolates of fungi that had been previously isolated from caves. The tests were carried out in vitro in 96-well-plates and the antimicrobial activity of the natural substances was evident through the absorbance changes in a microplate reader apparatus.

Results: The results showed that the natural antimicrobials with the best efficacy against microorganisms were the essential oils of Lamiaceae family (oregano and *Satureja* essential oils). Various specimens of *Juniperus* and *Laurus* showed medium efficacy, while essential oils and hydrosols from *Citrus, Foeniculum,* and *Salvia* presented low or no activity against the tested microorganisms. The current study demonstrated that herbal biocides may replace chemicals, thus, presenting intriguing products with significant prospects for commercial exploitation.

PW134 Occurrence of Antibiotics and Antibiotic Resistance Genes in Czech Wastewater Treatment Plants

Tereza Stachurová[1], Kateřina Malachová[1], Zuzana Rybková[1], Kateřina Svobodová[2]

[1]*University of Ostrava, Department of Biology and Ecology, Ostrava, Czech Republic*
[2]*Institute of Medical Biochemistry and Laboratory Diagnostics, Clinical Microbiology and ATB Centre, Prague, Czech Republic*

Background: Wastewater treatment plants (WWTPs) have been identified as a reservoir of antibiotic resistance bacteria (ARB) and antibiotic resistance genes (ARGs) that release into the environment. However, little is known about the persistence and diversity of ARGs during the wastewater treatment processes in WWTPs.

Objectives: Our study was aimed at the monitoring of relative abundance of beta-lactam resistance genes (blaTEM, blaNDM-1, blaKPC, blaOXA48) and tetracycline resistance gene (tetW) in different sampling points (nitrification tank and sedimentation tank) at two WWTPs influencing the Odra river and the Lučina river in the Czech Republic.

Methods: DNA was isolated from collected wastewater bacteria and the abundance of five ARGs was quantified using real-time PCR. Ampicilin resistant and non-resistant bacteria in wastewater were quantified by colony-forming unit determination. Antibiotic concentrations in wastewaters were measured using ultra-high performance liquid chromatography tandem mass spectrometry. The structure of bacterial populations was investigated using DGGE analysis of 16S rDNA amplicons.

Results: The content of four antibiotics, ampicillin, penicillin G, penicillin V, and tetracycline was determined in all wastewater samples. Tetracycline showed the highest concentration in nitrification tank (3.40 ng/L) and sedimentation tank (3.35 ng/L) of the WWTP influencing the Lučina river. The blaTEM gene showed the highest relative abundance out of the tested genes. From the sampling points its abundance was highest in the sedimentation tank of the WWTP influencing the Odra river.

PW135 Bacterial community and its relationship with pollutant removal in treatment wetlands

Kristjan Oopkaup[1], Jaak Truu[1], Marika Truu[1], Teele Ligi[2], Margit Kõiv-Vainik[2], Hiie Nõlvak[2]

[1]*Institute of Molecular and Cell Biology, Tartu, Estonia*
[2]*Institute of Ecology and Earth Sciences, Tartu, Estonia*

Background: Treatment wetlands (TWs) have been used over 50 years for purification of different types of wastewater. The treatment process is mostly mediated by microbial community, however, there are still many uncertainties about the structure, spatial distribution and activity of bacterial communities in TWs.

Objectives: The main aim was to characterize the bacterial community structure and diversity as well as relate the findings to the pollutant removal potential in different types of treatment wetlands.

Methods: A comparative study was performed based on the results of three experiments: (1) hybrid greywater treatment system (GTW); (2) horizontal subsurface flow filters of unplanted hybrid treatment wetland (MWTW); (3) created riverine treatment wetland complex (RTW). The bacterial community was characterized using 16S amplicon-based metagenomics and quantitative PCR.

Results: The origin of wastewater, treatment wetland type and the hydrologic mode affected the bacterial community structure of the treatment systems. Community diversity increased during the start-up period in MWTW and GTW systems over three-month, followed by the almost stable state up to the end of the experiment. Comparable bacterial community successional pattern was detected in the MWTW and GTW filter units with greater changes in community structure during the first operation months of the system.

The results suggest that the nitrogen removal occurs: (1) via coupled autotrophic and heterotrophic nitrification while the contribution of the aerobic denitrification is temporally variable; (2) via anaerobic denitrification, and nitrifier-denitrification; (3) in limited amount via ANAMMOX process with the highest potential in soils and sediments of the RTW complex.

PW136 Microbial communities response to the increase in temperature in Drinking Water Distribution Systems

Carolina Calero Preciado[1], Joby Boxall[1], Víctor Soria-Carrasco[2], Isabel Douterelo[1]

[1]*University of Sheffield, Civil and Structural Engineering, Sheffield, United Kingdom*
[2]*University of Sheffield, Animal and Plant Sciences, Sheffield, United Kingdom*

Background: Despite the presence of disinfectant residual, microorganisms are able to survive inside the pipes in Drinking Water Distribution Systems (DWDS). The majority of microorganisms thriving in DWDS live forming biofilms on the inner-pipes surfaces. However, the presence and mobilization of biofilms in bulk water has been associated with water quality problems. Climate change can lead to an increase of temperature in DWDS, which may affect microbial communities and their ecology, and thus alter water quality.

Objectives: To understand the effect of temperature increase on microbial communities composition in chlorinated DWDS.

Methods: For this study a full scale experimental DWDS facility was operated for 30 days at two different temperatures (16°C vs. 24°C). The facility allows simulating conditions in real DWDS and it has removable coupons inserted into the pipes that enable *in situ* analysis of biofilms. Water and biofilm samples were collected at the end of the experiment and key water physico-chemical parameters were measured to obtain information of the inner pipe environment. DNA from samples was extracted and the 16S rRNA and ITS rRNA genes were sequenced using Illumina, in order to characterize the bacterial and fungal diversity respectively.

Results: Differences in the composition of biofilm and planktonic microbial communities have been observed at 16°C and 24°C. Bacteria were the predominant taxa in water and biofilm samples. Fungal communities were primary present in biofilm samples. This change in microbial communities due to temperature increase can affect the water quality and compromise the distribution of safe water worldwide.

PW137 Differences in microbial nitrogen cycle in natural and managed tropical peatlands

Mikk Espenberg[1], Jaan Pärn[1], Kuno Kasak[1], Martin Maddison[1], Leho Tedersoo[2], Ülo Mander[1]

[1]*University of Tartu, Institute of Ecology and Earth Sciences, Department of Geography, Tartu, Estonia*
[2]*University of Tartu, Natural History Museum, Tartu, Estonia*

Background: Nitrogen cycle is one of the most important nutrient cycles and management of nitrogen is economically, ecologically and environmentally critical. Quantity and distribution of nitrogen is controlled through biogeochemical processes, however, the lack of knowledge regarding microbial processes governing nitrous oxide (N_2O) emissions is hindering climate-change impact estimations of tropical peatlands.

Objectives: The aim of this study was to assess the abundances of soil bacteria and archaea and their potential to perform different nitrogen transformation processes in tropical peatlands, and link these changes to N_2O and N_2 emissions from the peat.

Methods: The peat sampling from top soil was carried out in years 2013 to 2017 at natural and drained peatland sites of seven tropical regions (Myanmar, Borneo, Taiwan, Uganda, Florida, Pantanal, and French Guiana). Quantitative PCR was applied to evaluate the community sizes by quantifying the abundance of different genes. Physicochemical parameters and in situ N_2O and potential N_2 emissions were measured.

Results: Abundance of the bacterial 16S rRNA genes varied significantly between the sites, although archaeal 16S rRNA genes abundance was quite similar over most of the sites. We observed only low levels of bacterial *amoA* and ANAMMOX-specific 16S rRNA genes. Archaeal *amoA* and *nifH* genes were abundant across tropical peatlands. The *nirK* denitrifiers were significantly more abundant compared to *nirS*-type denitrifiers. Conversion of N_2O to N_2 was mainly controlled by microbes possessing *nosZI* genes in the wet sites and microbes possessing *nosZII* genes in the drained sites. In addition, *nrfA* gene-possessing microbes influenced the N_2O emissions.

PW138 Cultivation of a bacterium representing a previously unrecognized clade of the Nitrosomonas group; physiology, genomics, and distribution of a missing ammonia-oxidizer

Hirotsugu Fujitani[1;2], Syuta Kikuchi[3], Kento Ishii[3], Yuji Sekiguchi[1], Satoshi Tsuneda[2;3]

[1]*National Institute of Advanced Industrial Science and Technology, Tsukuba, Ibaraki, Japan*
[2]*Waseda University, Research Organization for Nano & Life Innovation, Tokyo, Japan*
[3]*Waseda University, Department of Life Science and Medical Bioscience, Tokyo, Japan*

Background: Members of the genus *Nitrosomonas* are well known as major nitrifiers in a variety of ecosystem; currently six subgenus-level clades are recognized. Our study revealed phylotypes possibly affiliating with an additional clade (unclassified cluster 1) of the genus could be retrieved from activated sludge; however, their ecophysiology and genomics remained unclear.

Objectives: In this study, we isolated a novel ammonia-oxidizing bacterium, strain PY1, representing the unclassified cluster and investigated key physiological and genomic characteristics, in comparison with representative ammonia oxidizers.

Methods: Doubling time, ammonium uptake rate, and maximum ammonium tolerance of strain PY1 were investigated. The genome of strain PY1 was extracted and the pair-end and mate-pair libraries were sequenced using Illmina MiSeq plastform. Raw reads merged with SeqPrep were assembled using SPAdes. Gene prediction and annotation were performed using the DDBJ Fast Annotation and Submission Tool.

Results: The doubling time was 34.7 h. The activity kinetics was investigated using the ammonium uptake rates. $K_{m(app)}$ value was 57.9 µM. The affinity for ammonia is highest among previously isolated AOB. Ammonia oxidizing activity was the lowest among all AOB and almost same levels as AOA. The draft genome of strain PY1 consists of four scaffolds (in total 2.9 Mbp) with mean G+C contents of 42.7%. The genome has 2,612 protein-coding sequences. Although strain PY1 contains genes likely related to oxidative stress, it is experimentally validated that the cells required catalase as H_2O_2 scavenger for cell growth. This implicates that strain PY1 coexists with other partners and defend from the stress.

PW139 Insights into Microalga and Bacteria Interactions of selected Phycosphere Biofilms Using Metagenomic, Transcriptomic, and Proteomic Approaches

Ines Krohn-Molt[1], Wolfgang Streit[1]

[1]*Institute of Plant Science and Microbiology, Microbiology and Biotechnology, Hamburg, Germany*

Background: It is well-known that microalga are associated with a microbiota. However, it remains unclear, if the associated microbiota in the phycosphere is specific for microalga strains and which role individual bacterial taxa play.

Objectives: Here we provide experimental evidence that *Chlorella saccharophila*, *Scenedesmus quadricauda*, and *Micrasterias crux-melitensis* are associated with unique and specific microbial populations.

Methods: Metagenome sequencing, binning approaches, secretome analyses in combination with RNA-Seq data implied fundamental differences in gene expression profiles of the microbiota associated with different microalga.

Results: Our analyses indicates that transcriptionally most active bacteria with respect to key genes commonly involved in plant–microbe interactions in *Chlorella* (Trebouxiophyceae) and *Scenedesmus* (Chlorophyceae) strains belong to the phylum of α-Proteobacteria. In contrast, in *Micrasterias* (Zygnematophyceae) phycosphere biofilm bacteria affiliated with the phylum of Bacteroidetes showed highest gene expression rates. Our data indicated that in general very common processes expressed in all three microbiomes with differences in few host interaction-related processes. Surprisingly, many of the transcripts matched genes that known to be of relevance in the interaction of higher plants. These included the infection-related secretion pathways und systems, biosynthesis of exoenzymes, modifying of carbohydrates, lipids, and flagella biosynthesis pathways. Especially the occurrence of flagella-related proteins confirmed by additional secretome studies. Similarly, flagella well known for plant infection and inducing plant innate immunity. Within this framework, our findings imply that some of the triggers and signals involved in the microbial interaction with higher plants are already of relevance in this evolutionary early system.

PW140 Metagenomic, phylogenetic and functional characterization of predominant endolithic green sulfur bacteria in the coral Isopora palifer

Shan-Hua Yang[1], Kshitij Tandon[2], Chih-Ying Lu[2], Sen-Lin Tang[2]

[1]*Tunghai University, Department of Life Science, Taichung, Taiwan*
[2]*Academia Sinica, Biodiversity Research Center, Taipei, Taiwan*

Background: Endolithic microbes in coral skeletons are known to be a nutrient source for the coral host. In addition to aerobic endolithic algae and *Cyanobacteria*, which are usually described in the various corals and form a green layer beneath coral tissues, the anaerobic photoautotrophic green sulfur bacteria (GSB) *Prosthecochloris* is dominant in the skeleton of *Isopora palifera*. However, due to inherent challenges in studying anaerobic microbes in coral skeleton, the reason for its niche preference and function are largely unknown.

Objectives: The objective of this study was to understand and clarify the role of the endolithic GSB in the skeleton of *Isopora palifera*.

Methods: We conducted multi-level approaches—including metagenomic, anaerobic cultivation, pigment analysis, ultrathin-section transmission electron microscopy, fluorescence in situ hybridization-nanoscale secondary ion mass spectrometry (FISH-NanoSIMS) and acetylene reduction assay (ARA)—to confirm the role of the endolithic GSB.

Results: This study characterized a diverse and dynamic community of endolithic microbes shaped by the availability of light and oxygen. In addition, anaerobic bacteria isolated from the coral skeleton were cultured for the first time to experimentally clarify the role of these GSB. This characterization includes GSB's abundance, genetic and genomic profiles, organelle structure, and specific metabolic functions and activity. Our results explain advantages endolithic GSB receive from living in coral skeletons, the potential metabolic role of a clade of coral-associated *Prosthecochloris* (CAP) in the skeleton, and the nitrogen fixation ability of CAP.

PW141 Exploring the bacterial reservoirs of the ICE SXT, vector of antibiotic resistances, in autochthonous communities from aquatic environments

Veronica Roman[1], Xavier Bellanger[1], Jesse Vänttinen[2], Marko Virta[2], Christophe Merlin[1]

[1]UNIVERSITY OF LORRAINE - CNRS, LCPME, UMR7564, Villers-lès-Nancy, France
[2]UNIVERSITY OF HELSINKI , Department of Microbiology, Helsinki, Finland

Background: SXT is an integrative and conjugative element (ICE) known to be involved in the dissemination of antibiotic resistance genes (ARGs) in *Vibrio* pathogens. If its dissemination is suspected to be linked to the consumption of antibiotics acting either as selectors or stimulators, its propagation in the environment is relatively unknown.

Objectives: Our aim was to map the occurrence of SXT in a continental aquatic environment and to characterize its environmental host range together with local environmental characteristics and pollutants.

Methods: A new set of primers was designed to monitor and/or quantify the SXT element by qPCR in complex microbial communities. The SXT bacterial host range was characterized by setting up and implementing a new epicPCR (Emulsion, Paired, Isolation and Concatenation PCR) approach. This new technic allows linking an SXT-specific DNA fragment to a phylogenetic marker (16S rDNA) by fusion PCR in single cells embedded in polyacrylamide beads. Fusion-PCR products from millions of cells are then sequenced by NGS for assigning a taxonomic group to the bacteria hosting SXT.

Results: Using qPCR, we could demonstrate that SXT is frequently found in wastewater treatment plant effluents
(~10^{-5}/16S) but also in various compartments of the continental aquatic environment such as rivers, pounds, aquaculture settings (10^{-4}-10^{-6}/16S). Contrary to what was anticipated with epicPCR, we found that SXT does not solely reside in *Vibrio*-like or *Proteus*-like bacterial hosts but also in locally adapted bacteria. This clearly establishes that the acquisition of ARGs in *Vibrio* pathogens probably follows complex dissemination paths including local autochthonous communities.

PW142 Characterization of bacteriophages for biocontrol of vibriosis caused by Vibrio alginolyticus

Rutinan Rattanaburee[1], Nattarika Chaichana[1], Decha Sermwittayawong[2], Natthawan Sermwittayawong[1]

[1]Prince of Songkla University, Department of Microbiology, Faculty of Science, Songkhla, Thailand
[2]Prince of Songkla University, Department of Biochemistry, Faculty of Science, Songkhla, Thailand

Background: *Vibrio alginolyticus* is one of the causative agents of vibriosis in marine fish and invertebrates. Disease outbreaks in aquaculture have been reported due to inappropriate antibiotics used in bacterial infection treatments. Phage therapy is one of the alternative solutions to control vibriosis causing by *V. alginolyticus* in aquaculture.

Objectives: To characterize lytic bacteriophages specific for pathogenic strains of *V. alginolyticus* and evaluate their performance to be used as biocontrol in *Galleria mellonella* larva model.

Methods: Host-range of bacteriophages were determined using *V. alginolyticus* and other *Vibrio* spp. Bacteriophages with broadest host-range were selected for morphological, phenotypic and genotypic characterization. The efficiency to decrease the mortality rates of *Galleria mellonella* larvae infected with pathogenic strains of *V. alginolyticus* were examined using single bacteriophage and bacteriophage cocktail.

Results: Three isolates of bacteriophages demonstrated the broadest host-range and could infect pathogenic strains of *V. alginolyticus*, *V. parahaemolyticus* and *V. vulnificus*. Their latent periods and burst sizes were ranging from 40 to 60 min, and 140 to 367 pfu/infected cell, respectively. Bacteriophage titers were slightly decreased after storage in artificial seawater for 180 days. They consist of dsDNA genome and could be classified as Siphoviridae family based on their morphology. We found that bacteriophage cocktail showed a significantly higher efficiency to reduce the mortality rate of *G. mellonella* larvae infected with pathogenic strains of *V. alginolyticus* than single bacteriophage. These results indicate that bacteriophage cocktail could be an environmental friendly alternative to control pathogenic *V. alginolyticus* infection in aquaculture.

PW143 Detection of bacterial and protozoal pathogens in ticks collected from dogs in eastern Austria

Michiel Wijnveld[1], Theresa Stelzer[1], Anna-Margarita Schoetta[1], Georg Duscher[2], Michael Leschnik[3], Hannes Stockinger[1], Gerold Stanek[1]

[1]Medical University of Vienna, Institute for Hygiene and Applied Immunology, Vienna, Austria
[2]University of Veterinary Medicine Vienna, Institute of Parasitology, Vienna, Austria
[3]University of Veterinary Medicine Vienna, Clinical Unit of Internal Medicine Small Animals, Vienna, Austria

Background: During a previous study ticks have been collected from dogs that were walked daily in a region in the east of Austria, which took place in a time period of 11 months (February-December) in 2008. From this collection, 174 ticks were selected at random, comprising of 75 *Dermacentor reticulatus* (47 female, 28 male), 44 *Haemaphysalis concinna* (21 females, 6 males and 16 nymphs) and 55 *Ixodes ricinus* (52 females, 3 males) ticks.

Objectives: The aim of the study was to assess the presence of tick-borne microorganisms in Austria.

Methods: To screen the ticks, the Reverse Line Blot (RLB) hybridization technique has been used, which is a technique that screens multiple ticks for the presence of DNA of multiple pathogens at once. Briefly, the RLB consists of a negatively charged nitrocellulose membrane that has up to 43 (geno)species specific oligonucleotides covalently bound to it. To screen for pathogen DNA, whole tick DNA extractions are subjected to several genus specific PCRs. The resulting PCR products are then loaded perpendicularly to the bound oligonucleotide probes using a miniblotter. Bound PCR products are visualized using chemiluminescence.

Results: In these ticks the DNA of the following pathogens was detected: *Anaplasma phagocytophilum*; *Borrelia afzelii*; *B. burgdorferi* sensu stricto; *B. garinii*; *B. lusitaniae*; *B. spielmanii*; *B. valaisiana*; *Candidatus* Neoehrlichia mikurensis; *Rickettsia helvetica*; *R. raoultii*; *Theileria* (*Babesia*) *microti*.

PW144 Synergistic biodegradation of cyanide from cassava mill effluent by consortium of nitrogen fixing microorganisms

Johnson Ndukwe[1]

[1]*University of Nigeria, Nsukka, Department of Microbiology, Nsukka, Nigeria*

Background: The indiscriminate discharge of cassava mill effluent (CME) poses serious environmental and public health threat. This work aimed at assessing the biodegradation potentials of indigenous microbes isolated from the soil and from root nodules of bean plant in alone in contrast with biodegradation potential when stimulated with rice husk.

Objectives: To isolate, screen, determine and compare biodegradatin potential of CME using a consortium of *Beijerinckia fluminensis* strain MA11 16S and *Pseudomonas putida* strain Rm1.

Methods: Seven set ups were mounted : Tray 1-7 CME contaminated soil alone (NC), Uncontaminated soil (U), CME contaminated soil and rice husk (NCR), CME contaminated soil + organisms (NCO), CME contaminated soil + rice husk + organism (NCRO), Artificially contaminated soil alone (AC), Artificially contaminated soil + organisms (ACO). The BOD, COD, CN, %TN, %TC were determined from the zero to the second week.

Results: At week zero the COD, CN, %TN and %TC were NCR (435 mg/kg, 0.160 mg/kg, 1.673%, 6.442%), NC (267.2 mg/kg, 0.168 mg/kg, 0.737%, 0.949%), NCO (271.5 mg/kg, 0.168 mg/kg, 0.737%, 0.949%), NCRO (438.2 mg/kg, 0.160 mg/kg, 1.673%, 6.442%), AC (249.6 mg/kg, 0.154 mg/kg, 0.659%, 0.304%), ACO (251.7 mg/kg, 0.154 mg/kg, 0.659%, 0.304%) and U (184.0 mg/kg, 0.056 mg/kg, 1.047%, 0.684%), respectively. Interestingly, the cyanide content (CN) was degraded up to 77% (0.037 mg/kg), 72% (0.043 mg/kg) and 48% (0.083 mg/kg) in the NCRO, ACO and NCO respectively, with least degradation occurring in the AC 2% (0.542 mg/kg), followed by NCR 18.1% (0.131 mg/kg) and NC 19.6% (0.135 mg/kg).

PW145 Fate of Pseudomonas aeruginosa and blaVIM in soil under selective pressure by copper and zinc

Gianuario Fortunato[1], Ivone Vaz-Moreira[1], Olga Nunes[2], Célia Manaia[1]

[1]*Universidade Católica Portuguesa, Centro de Biotecnologia e Química Fina , Porto, Portugal*
[2]*Universidade do Porto, LEPABE, Laboratório de Engenharia de Processos, Ambiente, Biotecnologia e Energia, Faculdade de Engenharia, Porto, Portugal*

Background: Besides the natural resistance to antimicrobials present in soil, antibiotic-resistant bacteria (ARB) and antibiotic resistance genes (ARGs) can be discharged in soil through irrigation or other agricultural practices, which can also be a source of other contaminants such as metals. It is hypothesized that metals may generate selective pressure enhancing the survival of bacteria and/or of their ARGs.

Objectives: This study aims to assess the survival of a multidrug-resistant *Pseudomonas aeruginosa* strain in soil contaminated with metals as well as the fate of the bla_{VIM} gene.

Methods: Assays were performed in sandy soil microcosms inoculated with *P. aeruginosa* strain H1FC49, in the presence and absence of potential selective pressures, generated by 20 mM $CuSO_4$ and $ZnSO_4$ or by $Cu(NO_3)_2$ and $Zn(NO_3)_2$. Microcosms were incubated at 25°C for 14 days and monitored weekly through the enumeration of *P. aeruginosa* on Cetrimide Agar + Nalidixic Acid and quantitative PCR of the housekeeping gene *ecf* and the acquired genes bla_{VIM} and *intI*1.

Results: The survival of *P. aeruginosa* in soil amended with copper and zinc nitrate was significantly lower than in a non-amended soil, while no significant difference was observed in presence of sulfate salts. After 14 days of incubation, in presence of the metal selective pressure, the *P. aeruginosa* housekeeping gene *ecf* decreased more than the acquired ARG bla_{VIM}. These results suggest that antibiotic resistance selection may occur mainly at the molecular rather than at the cellular level. The molecular mechanisms behind these variations will be further discussed.

PW146 Class 1 integron, marker of anthropic activity in Antarctica

Nicole Cid[1], Celia Lima[1], Gerardo Gonzales-Rocha[1], Helia Bello[1]

[1]*Universidad de Concepción, Laboratorio de Investigación en Agentes Antibacterianos, Chile*

Background: Antarctica, is considered one of the most pristine places on Earth; however, the constant exchange with the continent does not leave it outside of human impact that modify the diversity of the Antarctic microbiome and promoting genetic mobility. Integrons are one of the genetic platforms which have the ability to capture and express gene cassettes (GC). These gene cassettes harbor information for adaptability to the environment, including antibiotic resistance genes (ARG). These structures could be disseminated between autochthonous bacteria or discharged to the environment, facilitating its dissemination mainly by migratory birds or/and human activities. The main integron in clinic and environmental bacteria are those belong to class 1, and its presence has been proposed as a marker of pollution or anthropic impact.

Objectives: To quantify class 1 integrase gen (*intI1*) in soil samples of Antarctica recovered in zones with low and high human activity.

Methods: Eight soil samples from Fildes Peninsula, King George Island, were collected on February-March 2018 (54th Antarctic Scientific Expedition, ECA 54-INACH). The human activity was measured determining the coliform count, the relative quantification of *intI1* by qPCR using specific primers for class 1 integron and the DNA was extracted directly from the soil samples

Results: In the zones defined as “high human activity” the count of coliforms and the relative quantification of the *intI1* integrase gene were statistically significant higher ($p < 0.05$), supporting the idea that class 1 integron could be used as marker of anthropic activity.

PW147 A newly identified type III secretion-like chaperone and the Hfq RNA chaperone are required for cyanobacterial biofilm self-suppression

Rakefet Schwarz[1], Yevgeni Yegorov[1], Elad Nagar[1], Shaul zilberman [1], Eleonora Sendersky [1]

[1]*Bar-Ilan University, Faculty of Life Sciences, Ramat-Gan, Israel*

Background: Cyanobacterial biofilms are highly prevalent in the environment and are associated with substantial economic loss. We demonstrated that the planktonic nature of the cyanobacterium *Synechococcus elongatus* is a result of a self-inhibition mechanism, which depends on the deposition of a factor to the extracellular milieu. Inactivation of a gene encoding a homolog of the ATPase subunit of type II protein secretion or type IV pilus assembly systems (T2SE) impairs the inhibitory process and results in biofilm formation.

Objectives: Identification of additional components of the biofilm inhibitory mechanism.

Methods: A sequence-tagged random transposon library was employed for identification of additional components of the biofilm inhibitory mechanism. Newly identified proteins served for immunoprecipitation followed by MS to identify cellular targets of interaction. The exo-proteome was analyzed by MS. NMR served for structural analysis.

Results: We identified additional components of the biofilm inhibitory mechanism including the RNA-chaperone homolog Hfq and a highly conserved unique cyanobacterial protein (Se0862), thus far defined as "hypothetical". Immunoprecipitation experiments indicate a tripartite complex comprised of T2SE, Hfq and Se0862. Impairment of either one of the genes encoding T2SE, Hfq or Se0862, results in aberrant protein secretion and absence of pili, supporting involvement of these components in type II/type IV complexes. Structural analysis revealed homology of Se0862 to type III secretion chaperones of pathogenic bacteria, however, these secretion systems were not identified in cyanobacteria. We propose a unique modulation of type II/type IV complexes in cyanobacteria that employs an RNA-chaperone and a chaperone of type III systems.

PW148 Reductive anaerobic transformation and detoxification of sulfamethoxazole by sulfate reducing cultures

Weiying Ouyang[1], lorenz adrian[1]

[1]*Helmholtz-Zentrum Umweltforschung GmbH UFZ, Isotope Biogeochemistry, Leipzig, Germany*

Background: Sulfamethoxazole (SMX) is a veterinary antibiotic widely detected in the environment and routine sludge treatment cannot efficiently eliminate it. Anaerobic transformation is an important process for xenobiotics remediation in both natural and constructed systems.

Objectives: In our study, we investigated SMX-transforming cultures under anaerobic conditions, identifying an efficient way of SMX elimination and avoiding antibiotic resistance development.

Methods: We enriched and characterized mixed cultures for SMX transformation from sediment and digested sludge under different conditions.

Results: Highest activity of SMX transformation was found in sulfate reducing cultures. In contrast, by methanogenic low activity and by nitrate reducing no activity were seen. SMX exerted a toxic effect on sulfate reducing cultures, by inducing a longer lag phase for initiating transformation at higher SMX concentration. In sulfate reducing cultures, transformation products were identified showing that the isoxazole moiety of SMX was reduced. No SMX transformation in abiotic tests confirmed this reductive transformation was microbially mediated. The reduced transformation products were not re-oxidized to SMX by oxygen exposure, and their antibacterial activity was significantly decreased compared to that of SMX. However, reduction of SMX was not coupled to growth of specific bacteria. Population analysis in successive cultures revealed a strong community shift towards *Desulfovibrio* and *Desulfomicrobium* OTUs. Subsequently we observed similar reductive transformation of SMX with a pure strain of *Desulfovibrio vulgaris*. In summary, SMX can be reductively transformed by sulfate reducing bacteria and the anaerobic transformation is a detoxification process as the stable reduced products harbor less antibacterial activity than SMX.

PW149 The regulatory landscapes of copper-exposed Cupriavidus metallidurans CH34 and Caulobacter crescentus NA1000

Laurens Maertens[1;2], Rob Van Houdt[1], Jean-Yves Matroule[2]

[1]*SCK-CEN, Microbiology, Mol, Belgium*
[2]*UNamur, URBM, Namur, Belgium*

Background: *Cupriavidus metallidurans* CH34 is a β-proteobacterium originally isolated from a zinc decantation basin, able to grow in the presence of an impressive range of heavy metals. Despite thorough investigation, data on its regulatory features remain largely lacking.

In contrast, *Caulobacter crescentus* is an α-proteobacterium isolated from a freshwater environment. Displaying some tolerance to heavy metals, analysis of its copper resistance mechanisms has highlighted the importance of several genes. Like *C. metallidurans* CH34, there is currently little knowledge of its regulatory features during copper exposure.

In this study, we performed an in-depth analysis of these features using an adapted differential RNA sequencing (dRNA-Seq) protocol.

Objectives: Three objectives are principal: first, we aim to determine transcription start sites and transcript processing sites in the transcriptomes of copper exposed *C. metallidurans* CH34 and *C. crescentus* NA1000, with a focus on small RNAs. Second, genes with a role in the copper response will be detected using differential gene expression analysis. In a third analysis, we want to evaluate similarities in the regulatory features of both strains.

Methods: For both strains, mid-log phase cells were exposed to a sublethal concentration of $CuSO_4$, and total RNA was extracted. Primary and processed reads were labelled with different sequence tags, and fragmented cDNA was sequenced. The dRNA-seq reads were computationally analysed by edgeR and newly developed scripts.

Results: Transcription start sites and processing sites were accurately defined in both strains, leading to the discovery of several novel sRNA genes. In addition, interesting differential expression patterns were recognized.

PW150 Microbial community dynamics of a meadow contaminated with metals, natural and artifical radionuclides

Rogiers Tom[1;2], Williamson Adam[2], Rob Van Houdt[1], Natalie Leys[1], Nico Boon[2], Kristel Mijnendonckx[1]

[1]*SCK•CEN, Microbiology, Mol, Belgium*
[2]*UGent, Center for Microbial Ecology and Technology, Gent, Belgium*

Background: Historical discharges of phosphate and nuclear industry in Belgium have contaminated certain environmental sites with metals and radionuclides, possibly harmful for all organisms present.

Objectives: This study assesses the impact of metal and radionuclide contamination on the microbial community present in the Grote Nete river basin, a historical contaminated site in Belgium.

Methods: Eight soil samples with varying contamination levels were taken consecutively on one line perpendicular to the river and one control sample further in the field. General soil parameters like pH and moisture content were determined. ED-XRF and gamma spectroscopy were used to measure respectively metal elements and radionuclides. Furthermore, the microbial community was analysed via 16S rRNA microbial profiling.

Results: A clear metal and radionuclide contamination gradient was observed among the samples. The highest concentrations of metals are As and Zn ranging from respectively 9.60 to 2300 mg/kg and 49.40 to 3430 mg/kg. The most important radionuclide contamination originates from Ra^{226} with concentrations ranging from 19.2 to 3630 Bq/kg. In addition, a clear correlation between Ra^{226} and Cd contamination is observed. As expected, the moisture content is higher in highly contaminated samples, while a comparable pH of 5.08 to 5.49 was measured in all samples. The 16S microbial profiling resulted in over 7000 OTUs in total. Although no clear differences in the microbial community present in highly contaminated soils were observed, PCoA analyses clearly showed different groups depending on the level of contamination. This indicates that the contamination level does affects the microbial community structure.

PW151 Characterisation of lipase activity from surfactant-producing hydrocarbon-degrading Pseudomonads

Raji Bamanga[1], Yusuf Deeni[1], Andrew Spiers[1], Scott Cameron[1]

[1]*Abertay University, School of Science, Engineering and Technology., Dundee., United Kingdom*

Background: Lipases from Pseudomonads isolated from different environments can confer numerous advantages such as thermostability and solvent tolerance in its industrial application niche.

Objectives:

1. To isolate and characterise surfactant-producing bacteria from different soils.
2. To identify lipase-producing Pseudomonads among the surfactant-producing strains.
3. To characterise the lipase activity of selected Pseudomonad isolates.

Methods: A total of 1460 colonies from five sites were isolated using *Pseudomonas*-selective and non-selective nutrient media, screened for surfactant expression by drop-collapse assay and quantitative tensiometry. Sixty strains were selected and their phenotypes were assessed by a series of growth and behaviour-based assays. Furthermore, their tolerance to varying concentrations of heavy metals and temperature ranges were assessed. Their bioremediation potential was assessed by their ability to utilize diesel as a carbon source and their lipase production potentials were also assessed. Finally, the physiochemical properties of the lipase activity were assessed from selected strains.

Results: Of 168 strains found to express surfactants (24.7 mN/m – 26.7 mN/m, Tukey-Kramer HSD, $\alpha = 0.05$), 60 strains were selected and when investigated by Hierarchical cluster analysis demonstrated considerable phenotypic diversity. Eight out of the 60 strains could grow in high temperature (50 °C) while 35 of the 60 strains utilized diesel as a sole carbon source. Specific lipase activities of eight strains selected from the 60 ranged from 17.12 – 67.42 U/mg (soluble-fraction) and 30.45 – 130.06 U/mg (insoluble-fraction). Enzyme activity profiling was used to characterise pH, temperature and solvent tolerance as well as measuring enzyme kinetics of eight lipase-active lysates.

PW152 Occurrence of drug resistance in environmental strains of Bacteroides fragilis group bacteria

Sebastian Niestępski[1], Monika Harnisz[1], Ewa Korzeniewska[1], Adriana Osińska[1]

[1]*University of Warmia and Mazury in Olsztyn, Environmental Microbiology, Olsztyn, Poland*

Background: The anaerobic bacteria of the genus Bacteroides and related Parabacteroides include the group *Bacteroides fragilis* (BFG). These bacteria are potential pathogens that are frequently isolated from patients with anaerobic infections, often showing resistance to many antibiotics.

Objectives: The aim of this study was to assess the antimicrobial resistance of environmental strains of the *Bacteroides fragilis* group.

Methods: 123 BFG strains were isolated from human feces (nine samples), hospital wastewater, influent and effluent wastewater from a wastewater treatment plant (thirteen samples each), and from the lab rats feces (three samples). BFG strains were cultured on Bacteroides Bile Esculin (BBE) agar. Identification of isolates were carried out by PCR method (the presence of bfr gene) and MALDI-TOF MS method. The resistance to six antibiotic groups was evaluated by the agar dilution method on BBE agar.

Results: The percentage of resistant strains differed between the analyzed antibiotics and was determined at 97.56% for ciprofloxacin, 49.59% for erythromycin, 44.71% for ampicillin, 35.77% for tetracycline, 32.52% for amoxicillin/clavulanic acid, 26.83% for chloramphenicol, 26.01% for clindamycin, 11.38% for moxifloxacin, and 8.94% for metronidazole. The highest drug-resistance levels (up to 100% of isolates) and multi-drug resistance (94-97% of isolates) were observed in the strains isolated from influent and effluent wastewater samples. The present study showed that drug-resistant and multi-drug-resistant BFG strains are evacuated with treated wastewater to surface water bodies, which may pose potential health threats for humans and animals.

This work was supported by the National Science Centre (Poland) [grant No. 2016/23/N/NZ9/02167 and 2016/23/B/NZ9/03669].

PW153 Insights into metabolism of planctomycetes from Phycisphaerae class via comparative genomics

Alexander Elcheninov[1], Stepan Toschakov[1], Elizaveta Bonch-Osmolovskaya[1], Ilya Kublanov[1]

[1]Research Center of Biotechnology RAS, Department of Extremophiles Biology, Moscow, Russian Federation

Background: *Phycisphaerae* class is one of the phylogenetic branches within *Planctomycetes* phylum. Only 4 cultivated genera – *Phycisphaera*, *Algisphaera*, *Tepidisphaera* and *Sedimentisphaera* – were described to date. All cultivated species are carbohydrate-utilizers that generate energy via aerobic respiration and/or fermentation. But genetic determinants of these metabolic features so far have not been characterized.

Objectives: The aim of this study is the reconstruction of metabolic pathways, as well as phylogenetic and ecological diversity analysis, of cultivated and uncultivated *Phycisphaerae* representatives via comparative genomics.

Methods: Complete genome of *Tepidisphaera mucosa* was sequenced and assembled to a single chromosome. Genomes affiliated to *Phycisphaerae* and unclassified planctomycetal genomes were obtained from IMG and tested for completeness using CheckM. Almost complete genomes (>80% completeness) were used for phylogenetic analysis based on conserved single-copy proteins to determine “true” *Phycisphaerae* genomes. Final genome set were analyzed using IMG tools, dbCAN, UniProt to detect target genes.

Results: Based on phylogenetic analysis 22 selected *Phycisphaerae* genomes were divided into four groups which correlated with their habitats characteristics and metabolic features. All organisms were found to have a high hydrolytic potential due to numerous CAZymes genes – up to 200 in a single genome. All groups possessed determinants responsible for glycolysis, pentose-phosphate pathway, complete/partial TCA cycle and different types of fermentation. However, genes of respiratory ETC complexes were observed only in 2 of 4 groups. Thus *Phycisphaerae* is represented by highly diverse organisms based on their ecological and metabolic features.

The work was supported by Russian Science Foundation 17-74-30025.

PW154 Comparative genomic analysis of the representatives of genus Thermofilum: insights into the central metabolism and specific features of biosynthetic pathways

Kseniya Zayulina[1], Ulyana Piunova[2], Tatiana Kochetkova[1], Ilya Kublanov[1]

[1]Research Center of Biotechnology RAS, Department of Extremophiles Biology, Moscow, Russian Federation
[2]Lomonosov Moscow State University, Faculty of Bioengineering and Bioinformatics, Moscow, Russian Federation

Background: The family *Thermofilaceae* is a deeply branching phylogenetic lineage in *Crenarchaeota* phylum represented by a single cultivated genus comprising at present five isolates. All species are strictly anaerobic heterotrophs capable of growth on various organic compounds including complex polysaccharides and sugars. A common feature of all *Thermofilum* metabolism is the requirement of specific growth factors, presumably, the metabolites produced by co-habitating microorganisms.

Objectives: This study is focused on polysaccharides decomposition, central carbohydrate metabolism and distinctive features (lack of biosynthetic pathways) in *Thermofilaceae* anabolic pathways.

Methods: To reconstruct the metabolic pathways in *Thermofilaceae* we analyzed genomes of all cultivated isolates. The analysis of genome sequences was performed using IMG/MER system, KEGG and MetaCyc databases, dbCAN, Uniprot services. Growth experiments for auxotrophy detection were carried out in optimal conditions for each *Thermofilum* strain. The growth on the medium with substrate in the presence of other crenarchaea culture broth filtrate was used as a positive control.

Results: All *Thermofilum* genomes possess several tens CAZymes genes, involving in starch, glucomannan and other polysaccharides degradation. Central metabolism is represented by archaeal-type glycolysis and partial TCA cycle. For screening various metabolites serving as growth co-factors for *Thermofilum*s, genomes of co-habiting crenarchaeota were analyzed. Moreover, some pathways, like amino acid biosynthesis, pyrimidine biosynthesis, coenzyme A biosynthesis, pyridoxal 5'-phosphate biosynthesis are reduced or lacked in *Thermofilum* genomes. Presence of these anabolic pathways in co-habiting *Crenarchaeota* probably explains their significance for *Thermofilums* growth and metabolism.

The study is supported by the Russian Science Foundation grant # 018-44-04024.

PW155 The identification and functional genetic analysis of the pederin family gene cluster in alphaproteobacterium Labrenzia sp. PHM005

Dina Kačar[1], Pilar Rodríguez[2], Librada Cañedo[2], Virginia Rivera[1], Fernando de la Calle[2], Beatriz Galán[1], José Luis García[1]

[1]*CENTRO DE INVESTIGACIONES BIOLÓGICAS, Madrid, Spain*
[2]*PharmaMar S.A.*

Background: Polyketide synthases (PKS) are wide spread multi-enzymatic modular complexes that produce a large number of biomedical important natural products. Cytotoxic pederin family polyketides synthetized by trans-AT PKS are mostly associated with defense mechanisms in symbiotic bacteria. Few pederin or pederin-like biosynthetic gene clusters have been identified so far in symbionts of beetles, lichens and marine sponges. Recently, a free-living non-symbiotic bloom-forming Cyanobacteria and a marine Alphaproteobacterium, *Labrenzia* sp. PHM005, have been reported to produce pederin-like compounds.

Objectives: The functional characterization of the genes associated with a pederin analogue biosynthesis in *Labrenzia* sp. PHM005 will explain the assembly line of pederin-like compounds.

Methods: The PKS and nonribosomal peptide synthetase (NRPS) domains were analyzed using antiSMASH. The transcriptomic analysis was performed by qPCR. The production of the pederin analogue was analyzed using HPLC/MS. Gene deletions were done using the endonuclease I-*SceI* system.

Results: Here we show the identification and the characterization of a pederin-like gene cluster comprising three PKSs and tailoring enzymes responsible for the production of a pederin analogue. Based on this gene cluster we have proposed a plausible biosynthetic pathway for the production of the pederin analogue. This compound is synthesized both in rich and minimal media supplemented with glucose. Finally, we have generated a mutant lacking the starting PKS of the cluster, which impairs the pederin analogue production, demonstrating that the proposed cluster is responsible for pederin production in *Labrenzia* sp. PHM005.

PW156 Comparison of cave Actinobacteria communities according to hsp65 and 16S rRNA genes

Andrea Buresova[1;2;3], Jan Kopecky[1], Veronica Rodriguez-Nava[3], Marketa Sagova-Mareckova[1], Florian Vautrin[3], Lise Alonso[3], Yvan Moenne-Loccoz[3], Marek Omelka[4]

[1]Crop research institute v.v.i., Ecology and epidemiology of microorganisms, Prague, Czech Republic
[2]Charles University in Prague, Ecology, Prague, Czech Republic
[3]Université Claude Bernard Lyon 1, UMR CNRS 5557, Ecologie Microbienne, Villeurbanne, France
[4]Faculty of Mathematics and Physics, Charles University in Prague, Department of Probability and Mathematical Statistics, Prague, Czech Republic

Background: Caves are isolated and nutritionally limited environments thus representing extreme conditions in which only few bacterial taxa are able to survive. Actinobacteria were found to be highly abundant in bacterial communities of many caves. However, most studies focus on actinobacterial isolates or study their community using 16S rRNA, which is not sufficiently variable to distinguish related actinobacteria species. More precise identification of actinobacteria communities is necessary for searching novel or pathogenic members and understanding their role in caves.

Objectives: To determine actinobacterial community composition according to two gene markers. Identification of Actinobacteria members using hsp65 gene marker up to the species level.

Methods: We extracted DNA from four caves in France more or less affected by humans (Lascaux, Rouffignac, Reille, Mouflon) and using specific primers for 16S rRNA gene (for bacteria) and hsp65 (for Actinobacteria) we sequenced the related genes by Illumina MiSeq. The sequences were processed by MOTHUR and distance matrices were compared to assess the community composition differences between the caves according to the both gene markers. Database of hsp65 genes was created to classify Actinobacteria members into the species.

Results: Our results showed that both gene markers specifically describe differences of the Actinobacteria communities between the caves. According to hsp65 gene marker, pathogenic actinobacterial members were identified in caves which confirmed the potential of this gene marker for monitoring of uncultured Actinobacteria strains in environment.

PW157 A new multicomponent regulatory system that controls carbon catabolite repression of the anaerobic catabolism of aromatic compounds in Azoarcus sp. CIB

Unai Fernandez[1], J. Andres Valderrama[2], Helena Gómez Álvarez[2], Zaira Martín Moldes[2], Gonzalo Durante Rodríguez[2], Eduardo Díaz[2]

[1]Biological Research Center, Departamento de Biotenología Microbiana y de Plantas, Madrid, Spain
[2]Biological Research Center, Madrid, Spain

Background: Aromatic compounds are major environmental pollutants. Anaerobic biodegradation of aromatics usually involves the well-known benzoyl-CoA central pathway (*bzd* genes). The beta-Proteobacterium *Azoarcus* sp. CIB has been used as model system to study the effector-specific regulation of the *bzd* genes. However, an efficient biodegradation also depends on global regulators. We expand previous studies towards the characterization of the first multicomponent regulatory system that controls carbon catabolite repression (CCR) of the *bzd* genes in bacteria.

Objectives: To unravel the multicomponent regulatory system that mediates *bzd* carbon catabolite repression by some organic acids, e.g., succinate, in *Azoarcus* sp. CIB.

Methods: Gene expression studies were performed by qRT-PCR and β-galactosidase assays of P_N::*lacZ* fusions, in the wild-type CIB strain and in *accS*, *accR* and *accT* null mutants, as well as in recombinant *E. coli* cells expressing reporter fusions. Different genetic and biochemical assays were carried out to characterize the AccS and AccT proteins.

Results: CCR of the anaerobic degradation of benzoate is mediated by the *acc* gene cluster in *Azoarcus* sp. CIB. The AccR response regulator that represses the expression of the *bzd* genes in the presence of succinate is activated by a multidomain sensor histidine kinase (AccS) whose cytoplasmic autokinase domain responds to the redox state of the host cell. A periplasmic solute-binding protein (AccT) might recognize organic acids and control autophosphorylation of the membrane-bound AccS. The *acc* cluster encodes a new regulatory circuit that is conserved in several β-proteobacteria where it may control their global metabolic state.

PW158 Unraveling a specific cyclohexane carboxylic acid degradation pathway in bacteria

David Sanz[1], eduardo díaz[1]

[1]*The Biological Research Center, Microbial and plant biotechnology, Madrid, Spain*

Background: Cyclohexane carboxylic acid (CHCA) is widely distributed in nature coming from lignin-containing materials and is present at high concentrations as an environmental pollutant in wastewater from petrochemical plants. Bacterial biodegradation is an eco-friendly strategy to remove CHCA from contaminated environments. Anaerobic CHCA degradation has been described so far in the phototrophic *Rhodospseudomonas palustris* and in some obligate anaerobes where it funnels to the anaerobic benzoate degradation pathway. Here we characterize for the first time a specific CHCA degradation pathway that is present in several bacteria.

Objectives: Characterization of a CHCA degradation pathway in the β-Proteobacterium *Azoarcus* sp. CIB.

Methods: Gene expression and transcriptional organization of *bad* genes were analysed by RT-PCR. *lacZ* translational fusions were used to study the BadR-specific regulation of the *bad* operon in *E. coli.* Biochemical approaches and *in vitro* interaction assays, e.g., gel-retardation, were used to characterize the BadR-dependent repression of *bad* genes.

Results: The *bad* cluster encodes the aerobic and anaerobic CHCA degradation pathway, whose expression and biochemical steps are different to those of the bzd pathway for anaerobic degradation of aromatic compounds in *Azoarcus* sp. CIB. The *bad* genes are present in several facultative anaerobic bacteria suggesting that the CHCA degradation ability is more widely distributed in bacteria than previously thought. The transcriptional organization and regulation of the *bad* genes by the BadR repressor has been characterized. A mobilizable bad synthetic cassette has been engineered and used to efficiently expand CHCA degradation to different environmentally relevant bacteria unable to degrade this compound.

PW159 Temperature effect on biofilm formation in Pseudomonas aeruginosa

Suran Kim[1], Hyeon-Ji Hwang[1], Joon-Hee Lee[1]

[1]College of Pharmacy, Pusan National University, Department of Pharmacy, busan, Korea, Rep. of South

Background: In response to external cues, bacteria form biofilm that is very protective life mode and adapt to environmental changes. *Pseudomonas aeruginosa*, an opportunistic human pathogen also adopts biofilm as a protective life mode against environmental challenges. During infection into human, *P. aeruginosa* experiences a big difference in temperature between the environment and the human body. This temperature change may act as a cue to *P. aeruginosa* for the biofilm formation.

Objectives: In this study, we investigated the effects of temperature on the *P. aeruginosa* biofilm formation.

Methods: We measured the biofilm formation of *P. aeruginosa* at different temperatures (20℃, 25℃, 30℃, and 37℃) in static- and flow cell-systems. We also measured intracellular cyclic di GMP (c-di-GMP) levels by using reporter (*cdrA$_p$-lacZ* fusion) or specific fluorescence dye.

Results: Our results demonstrated that the lower the temperature, the better the biofilm formed by *P. aeruginosa.* Consistently, the level of intracellular c-di-GMP was higher at lower temperatures. In conclusion, in the temperature range between 20℃ and 37℃, the lower the temperature, the more the c-di-GMP is synthesized, and the better the biofilm is formed by *P. aeruginosa*.

PW160 High temperature promotes cell-to-cell plasmid transformation in solid-air biofilms of Escherichia coli

Mayuko Hashimoto[1], Haruka Hasegawa[1], Sumio Maeda[1]

[1]*Nara Women's University, Graduate School of Humanities and Sciences, Nara, Japan*

Background: Horizontal gene transfer (HGT) between bacterial cells contributes to bacterial adaptation to variable environments. Recently, variations in conventional HGT mechanisms (transformation, transduction, and conjugation) and other new mechanisms have been proposed. We previously reported that DNase-sensitive horizontal transfer of nonconjugative plasmids occurred between two *Escherichia coli* strains in co-culture, termed "cell-to-cell plasmid transformation"[1,2].

Objectives: In this study, we aimed to investigate the effect of temperature on cell-to-cell plasmid transformation in a simplified system.

Methods: Cell-to-cell plasmid transformation experiments in solid-air biofilms or in liquid culture were performed by co-culturing a laboratory strain of *E.coli* harboring a plasmid with the same strain harboring another plasmid possessing a different antibiotic-resistance gene and a different replication origin. Only conjugation- and phage-free strains and plasmids were used. Transformants were detected as double-resistant colonies on agar plates.

Results: We found that temperatures of >40°C promoted plasmid transfer. In the best strain-plasmid combination and conditions tested, plasmid transfer frequency reached 10^{-7}–10^{-6}/cell. The transfer frequency in solid-air biofilms was 10–10^2 times greater than that in liquid culture, suggesting that a biofilm environment is best-suited for this plasmid transfer to occur. The plasmid transfer was DNase-sensitive, demonstrating that this plasmid transfer was due to transformation. Because high temperatures of >40°C are common in avian intestines, our results suggest that such intestines may be a suitable location where frequent HGT by cell-to-cell transformation occurs.

[1] Etchuuya, *et al*. (2011) *PLoS One* 6 :e16355

[2] Hasegawa, *et al*. (2018) *Front .Microbiol*. 9:2365

PW161 Antibiotics effects on carbon and nitrogen uptake by aquatic microbial communities - a single cell approach

Federica Calabrese[1], Stryhanyuk Hryhoriy[1], Brauns Mario[2], Richnow Hans Hermann[1], Niculina Musat[1]

[1]*Helmholtz Centre for Environmental Research, Isotope Biogeochemistry, Leipzig, Germany*
[2]*Helmholtz Centre for Environmental Research - UFZ, River Ecology, Magdeburg, Germany*

Background: The increased use of antibiotics in the past decades to treat human and animal infections, to prevent bacterial diseases in agriculture and to promote growth of poultry, beef and pigs in animal farms resulted in the contamination of natural ecosystems. The diffusion of antibiotics in the environment, especially in natural water systems, is considered an immediate threat due to the development and spread of antibiotic resistance genes with direct risk to human health. On long term, however, the effect of antibiotics on the functioning and ecology of natural microbial communities with further major implications in biochemical cycling of elements (e.g. remineralization, organic contaminant degradation and ecosystem functioning) remains presently not understood.

Objectives: Here we aimed to determine the effects of different concentrations of antibiotics on carbon and nitrogen uptake by single-cells of native aquatic microbial communities. Water samples were collected from pristine sites and from waste water effluents.

Methods: We used stable isotope labelling with ^{13}C-acetate and ^{15}N-ammonium and spiked the naturally collected surface waters which were further incubated for 24h at in situ temperature conditions. Following incubation experiments samples were analyzed by nano-scale secondary ion mass spectrometry (NanoSIMS).

Results: Our preliminary results shows that i) the antibiotic presence decrease considerably the uptake of C and N by native microbial cells ii) the higher the antibiotic concentration the higher the impact on the C and N uptake and iii) the antibiotic presence seems to select for particular microbial groups which stay highly metabolically active.

PW162 Metagenomic analysis of open-air and indoor spent fuel storage ponds at Sellafield, UK

Sharon Ruiz Lopez[1], Lynn Foster[2], Nick Cole[2], Hokyung Song[3], Jonathan Adams[4], Jonathan Lloyd[1]

[1]*The University of Manchester, SEES, United Kingdom*
[2]*Sellafield Ltd, Seascale, United Kingdom*
[3]*Seoul National University, Korea, Rep. of South*
[4]*Cranfield University, Wharley End, United Kingdom*

Background: Nuclear power is an important energy source that can compensate carbon emissions from fossil fuel power plants. However, processing radioactive waste from nuclear plants is a significant challenge. The current treatment prior to final geological disposal involves wet storage of spent fuel in designated ponds, and microbial colonisation of these ponds can complicate plant operation.

Objectives: To identify the key microbes that colonise hydraulically interlinked spent fuel storage ponds at Sellafield, UK

To assess the biological function by metagenomic sequencing and analysis.

Methods: A series of samples were collected and analysed using next generation (Illumina) sequencing. Samples were taken from the facility´s indoor Fuel Handling Plant (FHP) pond (feeding head tank, main and subponds), and also from the open-air First Generation Magnox Storage Pond (FGMSP).

Results: 16S rRNA gene sequencing revealed that the FHP is colonized mainly by Bacteria (99%), affiliated with species of *Curvibacter, Rhodoferax, Sphingomonas* and *Roseococcus,* in addition to the hydrogen-oxidising bacterium *Hydrogenophaga*. In contrast the open-air FGSMP pond contained species of *Hydrogenophaga, Nevskia,* and *Roseococcus*, and also photosynthetic cyanobacteria (*Pseudanabaena*). The most abundant genes were associated with carbohydrate and protein metabolism, cell wall and capsule synthesis, stress responses and respiration. Genes involved in respiration were also more abundant in the indoor pond microbiome, including genes underpinning hydrogen metabolism, whilst photosynthesis genes were more abundant in the open-air ponds. These datasets give valuable insight into the microbial communities inhabiting nuclear storage facilities, the metabolic processes that underpin their colonisation and can help inform appropriate control strategies.

PW163 Characterization of the porduction and structure of dextran synthesized by Lactobacillus hordei TMW 1.1822 at different pH values

Jonas Schmid[1], Rudi F. Vogel[1], Frank Jakob[1]

[1]*Lehrstuhl für Technische Mikrobiologie, Technische Universität München, Freising, Germany*

Background: Bacterial dextrans play a major role in the formation of biofilms and are exclusively produced by lactic acid bacteria (LAB) via the release of dextransucrases (EC 2.4.1.5), which use sucrose as substrate for the dextran polymerization. They are composed of α-D-glucose monomers, which are linked via α–1→6 glycosidic bonds. The molecular weight of dextrans is versatile and particularly depends on the dextransucrase activity, the substrate concentrations and the pH.

Objectives: Since the pH is variable in the extracellular milieu of LAB, we wanted to systematically establish relationships between the dextran production pH and the amount and size of synthesized dextrans.

Methods: In this work, we used *Lactobacillus (L.) hordei* TMW 1.1822 for the controlled production of dextran in buffer at different pH to analyze differences in yields, sizes, molecular masses and shapes.

Results: We recovered the released dextransucrase of *L. hordei* in buffer and were able to directively modify the mass and size of the dextran by applying different production pH. The dextran was composed of fractions of two molecule shapes, whose ratios vary in dependence of the production conditions. *L. hordei* has been isolated from the traditionally fermented beverage water kefir, in which LAB, bifidobacteria and yeasts build up biofilms in dextran matrices (kefir granules). Since the physicochemical properties of EPS strongly depend on their molecular weight and molecule shapes, our systematic study gives the opportunity of new insights into the influences of environmental factors on the properties of biofilm forming macromolecules and potential triggering mechanisms.

PW164 Identification of a novel type of dextransucrase in water kefir adapted Lactobacillus hordei TMW 1.1822

Julia Bechtner[1], Rudi F. Vogel[1], Frank Jakob[1]

[1]*Lehrstuhl für Technische Mikrobiologie, Technische Universität München, Freising, Germany*

Background: Dextransucrases (EC 2.4.1.5) are enzymes belonging to the glycoside hydrolase family 70 (GH70) using sucrose as sole substrate to form α-glucans (dextran) upon fructose release. These enzymes are produced by lactic acid bacteria (LAB) and are diverse regarding their size and amino acid composition. Dextran producers are commonly isolated from sucrose-rich environments. As such, water kefir, fermented by diverse LAB species, is a promising reservoir for LAB harboring yet unknown dextransucrases. Within the water kefir microbiota, *Lactobacillus (L.) hordei* was found to be one of the dominant LAB species.

Objectives: Investigation of the genomic adaptation of *L. hordei* TMW 1.1822 to the water kefir environment with special focus on the identification and subsequent analysis of putative dextransucrases.

Methods: *L. hordei* TMW1.1822 was whole genome sequenced and the protein sequence of a predicted dextransucrase was computionally analysed. Expression and release of this dextransucrase into water kefir medium was analysed by SDS-PAGE.

Results: Indeed, genomic adaption of *L. hordei* TMW 1.1822 to the environment manifested primarily in its carbohydrate metabolism. As part of this, a dextransucrase was identified, enabling extracellular sucrose metabolism. Expression and release of this enzyme were proven. Interestingly, the dextransucrase accumulated in the cytoplasm of the strain, but was only released in the presence of sucrose. Apart from the characteristic GH70 catalytic core and glucan-binding domains, the amino acid sequence revealed no known type of signal motif. This indicates an unusual sucrose-induced release mechanism displaying once more the adaption of *L. hordei* TMW 1.1822 to its habitat.

PW165 Associations Between Reduced Susceptibility to Antibiotics and Non-Antibiotic Antibacterials in Natural and Clinical Isolates of Escherichia coli

Anna Bischofberger[1], Richard Allen[1], Michael Baumgartner[1], Alex Hall[1]

[1]*Institute of Integrative Biology, Pathogen Ecology, Department of Environmental Systems Sciences, Zurich, Switzerland*

Background: Many factors influence the emergence of antibiotic resistance (AR): In non-laboratory environments, bacteria encounter non-antibiotic antibacterials (NAAs), e.g. disinfectants and heavy metals. Resistance evolution to NAAs has been observed for various strains and conditions, with underlying mechanisms being thought to be often the same as known for AR (e.g. increased expression of unspecific efflux pumps, degradation enzymes).

Objectives: We tested whether resistance to NAAs co-occurs with resistance to clinically relevant antibiotics in natural and clinical isolates, and what are the evolutionary mechanisms explaining such associations. We assessed the effect of NAA-adaptation on AR.

Methods: We determined the susceptibility of 93 natural and clinical *E. coli* isolates to five NAAs (benzalkonium chloride, cadmium, sodium-dodecyl-sulfate, triclosan, zinc) by broth dilution and mapped the results against existing ABR profiles of these isolates. We experimentally evolved isolates in presence of NAAs and assessed the effect on antibiotic susceptibility. We tested whether observed associations could be explained by the same allele affecting both types of resistance (knockout experiments), or by carriage of multiple resistance alleles (heavy metal and biocide resistance and AR gene data inferred from genome sequences).

Results: We found that resistance to NAAs is not generally associated with ABR, but we detected a significant positive association between benzalkonium chloride and triclosan resistance; resistance to both NAAs is significantly associated with chloramphenicol resistance. We found that adaptation to triclosan influences ABR (depending on antibiotic), but not adaptation to benzalkonium chloride. Our results show how exposure to NAAs can promote AR.

PW166 Detection of Microbial Pathogens in Ixodes ricinus Ticks Collected in Austria and Impact of the Chosen Method

Anna-Margarita Schoetta[1], Michiel Wijnveld[1], Hannes Stockinger[1], Gerold Stanek[1]

[1]*Institute for Hygiene and Applied Immunology, Center for Pathophysiology, Infectiology and Immunology, Medical University of Vienna, Vienna, Austria*

Background: Ticks are the second most common hematophagous vectors after mosquitos and can transmit a huge number of various pathogens that can lead to human disease. Therefore surveillance and knowledge of the microorganisms harboured by ticks in certain regions is of great importance.

Objectives: Aim of the study was to determine the prevalence and presence of tick-borne microorganism in different locations of Austria that can affect human health. Moreover, different molecular detection approaches were compared.

Methods: Ticks were screened with the reverse line blot (RLB) hybridization method which allows for detection and identification of multiple microorganisms such as *Anaplasma*/*Ehrlichia* spp., *Candidatus* Neoehrlichia mikurensis, *Babesia* spp., *Rickettsia* spp., *Borrelia burgdorferi* sensu lato, and *Coxiella burnetti*. Different variations of the RLB were used for detection of *B.burgdorferi* sensu lato and *Rickettsia* spp. to investigate the impact of the chosen method.

Results: The pathogen with the highest prevalence detected in the tested ticks was *Borrelia burgdorferi* sensu lato in 25.6% with *B.afzelii* being the most frequently detected species. *Rickettsiae* spp. represented the pathogen group with the second highest prevalence with an infection rate of 16.8% of the ticks. *Candidatus* Neoehrlichia mikurensis, *Babesia* spp. (*B.venatorum*, *B.divergens*, *B.microti*) and *Anaplasma phagocytophilum* have been detected in 4.5%, 2.7% and 0.7%, respectively. No *Coxiella burnetti* positive ticks were found. We also showed that different detection approaches can considerably influence epidemiological results.

PW167 Antibiotic resistance of bacetria responsible for foodborne diseases derived from poultry farms with different levels of antibiotics use

Katarzyna Kosznik-Kwaśnicka[1], Łukasz Grabowski[2], alicja nowak-zaleska[2], agata jurczak-kurek[3], agnieszka necel[2], aleksandra dydecka[2], alicja węgrzyn[1]

[1]Institute of Biochemistry and Biophysics Polish Academy of Sciences, Laboratory of Molecular Biology, Gdańsk, Poland
[2]Faculty of Biology University of Gdańsk, Department of Molecular Biology, Gdańsk,
[3]Faculty of Biology University of Gdańsk, Department of Molecular Evolution, gdańsk, Poland

Background: Poultry meat is currently the most popular type of meat to consume in countries all around the world and its consumption is constantly growing. However, it is also commonly responsible for foodborne diseases like salmonellosis or campylobacteriosis. Use of antibiotics on farms led to drug resistance development in pathogens causing those illnesses. Birds from backyard farms are considered to be "healthier" as animals are being bred without antimicrobial additives. However, those birds are often being fed leftovers, which may result in various bacteria colonizing their gastrointestinal tract.

Objectives: The aim was to compare the level of antibiotic resistance and the presence of drug-resistance genes in bacteria responsible for food poisoning (*Salmonella enterica, Campylobacter sp., Escherichia coli*) isolated from poultry of farms with different levels of antibiotic use.

Methods: Bacteria were isolated from birds feces and cloaca swabs according to ISO norms. Identification of microorganisms was conducted by biochemical testing and 16S DNA sequencing. Resistance to antibiotics was analysed by disc diffusion method. Furthermore, PCR were performed in order to analyse the presence of antibiotic-resistance genes.

Results: We have observed that *Salmonella* sp. and *Campylobacter* spp. were most commonly present in samples collected from backyard farms, while samples from industrial farms contained mainly various strains of *E. coli*. Therefore, public opinion about poultry from private farms being "healthier" may be misleading. We have also observed that isolated pathogens were less susceptible to antibiotics, mainly tetracycline and ampicillin.

PW168 compartmentlization of class 1 integrons and incP-1 plasmids in a river ecosystem impacted by anthropogenic activities

Christophe Merlin[1], Magali De la Cruz Barron[1], Xavier Bellanger[1]

[1]*UNIVERSITY OF LORRAINE - CNRS, LCPME UMR7564, VILLERS-LES-NANCY, France*

Background: As final recipient of most anthropogenic wastes, aquatic ecosystems occupy a key position in the dissemination of antibiotic resistant genes (ARGs). Once in the aquatic ecosystems, ARGs can persist, transfer between bacteria, and accumulate in reservoirs, which may represent a risk for human health if they are re-mobilized.

Objectives: The aim of this work was to study the fate of ARGs in the different compartments (water, suspended materials, sediments) of a river ecosystem impacted by past metallurgic activities (Orne River, France), using two mobile genetic elements (MGEs) as proxies, namely class 1 integrons and IncP-1 plasmids.

Methods: Raw water, suspended materials (collected with a continuous flow field centrifuge), and sediments (collected by coring) of the Orne River (Eastern France) were sampled repeatedly from 2015 to 2018. Abundances of class 1 integrons and IncP1 plasmids were estimated by qPCR from community DNA extracts.

Results: In the Orne River, the entry of both MGEs in the ecosystems appeared diffuse rather than localized, and their respective abundances are mainly driven by parameters related to seasonality and hydrodynamics. Both MGEs, but mostly IncP-1 plasmids, are differentially enriched in suspended materials and the top layers of sediments (recent deposits) compared to raw water, which likely result from the increased cell adhesion properties provided by the presence of conjugative plasmids. In sediments, MGEs form reservoirs with abundances that decrease with depth, but also negatively correlate with the concentration of trace metal elements and polycyclic aromatic hydrocarbons, suggesting that toxicity may dominate over co-selection processes.

PW169 Identification of extracellular DNA in P.fluorescens SBW25 air-liquid interface biofilms.

Olena Moshynets[1], Andrew Spiers[2]

[1]*Institute of Molecular Biology and Genetics of National Academy of Sciences of Ukraine, Kiev, Ukraine*
[2]*Abertay University, Dundee, United Kingdom*

Background: Extracellular DNA (eDNA) is now recognised as an important structural component of biofilms where it also enables horizontal gene transfer and local adaptation. Although biofilm–formation by the model rhizosphere bacterium *Pseudomonas fluorescens* SBW25 has been extensively investigated, the presence of eDNA in the cellulose-matrix–based biofilms this strain produces has not been reported as it has for related pseudomonads including the plant pathogen *P. syringae* and the opportunistic human pathogen *P. aeruginosa*.

Objectives: To investigate eDNA in SBW25 biofilms.

Methods: Combined biofilm assay, CLSM, ultracentrifugation, TEM.

Results: eDNA was observed in both the weak and poorly-attached Viscous Mass (VM) and robust and well-attached Wrinkly Spreader (WS) air-liquid (A-L) interface biofilms by CLSM. This material could be precipitated from biofilm cell-free supernatant samples which demonstrated that WS biofilms had two-fold–higher levels than VM biofilms. DNAse treatment effected both types of biofilm development and reduced the strength and attachment levels when added to mature VM and WS biofilms. Testing with exogenous DNA suggests that high molecular weight (HMW) DNA is involved in both strength and attachment, perhaps by surface conditioning and interactions with the primary cellulose matrix common to both biofilms. HMW eDNA could be isolated directly from biofilm supernatants whereas two different HMW size fractions could be isolated from outer membrane vesicles, suggesting that eDNA persistence and degradation in SBW25 biofilms is complex, or perhaps that eDNA fractions may play different roles in biofilm development, protection and adaptation.

PW170 Biomonitoring of Faecal Indicating Bacteria in Rivers using Freshwater Sponges

Allison Cartwright[1], Joerg Arnscheidt[2], James Dooley[2], Chris McGonigle[1]

[1]Ulster University Coleraine Campus, Geography and Environmental Sciences, Coleraine, United Kingdom
[2]Ulster University Coleraine Campus, Biomedical Sciences, Coleraine, United Kingdom

Background: Filter feeding sponges (Porifera) continually uptake aquatic bacteria. Sponges concentrate bacteria from the water and retain them within their body, thus allowing the detection and quantification of specific bacterial groups from sponge samples. The extended contact of sponges with aquatic bacteria could allow for better determination of bacterial water quality.

Objectives: This study had two objectives:

Investigate sponges' ability to retain faecal indicating bacteria (FIB) with different relative abundances of *Escherichia coli* and *Enterococcus faecalis*.

Compare the concentration of coliforms and enterococci in sponges and water samples with different proximity to a wastewater treatment facility.

Methods: For objective 1 sponges (Ephydatia fluviatilis) were labratory grown from suface-sterilised gemmules. These juvenile sponges were exposed to different ratio abundances of E. coli and E. faecalis. For objective 2 sponge samples were collected from the Cavan River, Ireland with different proximity to a wastewater treatment outlet (35 m upstream; 100 m, 300 m, 900 m downsteam). All sponges were homogenised and serial diluted before being plated onto selective MacConkey agar No 3 (coliforms) and Slanetz & Bartley media (enterococci) for quantification.

Results: Sponges retained E. coli and E. faecalis regardless of their abundance of exposure. Sponges could be used to detect coliforms and enterococci from river water. The abundance of FIB varied along the river but was not related to the wastewater outlet as this was not the only pollution input. These results provide evidence that freshwater sponges can be used as a biomonitor for FIB in rivers.

PW171 monitoring the biological activity of different composting processes: humification, germination and respirometric indexes

Macarena del Mar Jurado Rodríguez[1], francisca suárez-estrella[1], maría josé lópez[1], juan antonio lópez-gonzález[1], joaquín moreno[1]

[1]*Universidad de Almería, Biology and Geology, Almería, Spain*

Background: One of the most important aspects to evaluate the quality and viability of the product obtained after the treatment of biodegradation of organic waste is the determination of its state of maturity. This term is considered as the sum of two key concepts: biological stability and humification, both related to the degree of decomposition of organic matter due to the action of microorganisms.

Objectives: The aim of this work was to study parameters related to the state of decomposition, humification and phytotoxicity of 5 types of organic waste subjected to composting. The evolution of these parameters throughout the composting could offer a deeper insight into the mechanisms involved in the biodegradation of the organic matter.

Methods: To carry out this work, compost samples collected from 15 facilities dedicated to the treatment of Urban Solid Waste (USW), Vegetable Wastes (VW), Sewage Sludge (SS), Agrifood Waste (AW) and olive mill wastes “Alpeorujo” (ALP) were used. The consumption of O2, the germination index (GI) and the content of the different humic and fulvic fractions were analyzed.

Results: As a result of these analyzes, a reliable image of the state of maturity of the samples was obtained, which was very useful to evaluate their quality against a possible agronomic application, as well as to achieve a better understanding of the composting process based on the raw materials used in each case. In fact, a significant positive correlation was detected between phytotoxicity parameters and biodegradability, agreeing with the degree of humification of the samples.

PW172 Impact of synthetic silver nanoparticles on biofilm microbial community antibiotic resistome in wastewater treatment system

Jaak Truu[1], Marika Truu[1], Hiie Nõlvak[2], Teele Ligi[1], Kristjan Oopkaup[1], Kaja Kasemets[3]

[1]*University of Tartu, Faculty of Science and Technology, Tartu, Estonia*
[1]*University of Tartu, Faculty of Science and Technology, Tartu, Estonia*
[3]*National Institute Of Chemical Physics And Biophysics, Tallinn, Estonia*

Background: Release of synthetic nanoparticles (SNP) from consumer and household products into wastewater and further into the wastewater treatment system may affect the system's microbial community that may lead to altered treatment efficiency and increased release of antibiotic-resistant bacteria into the environment.

Objectives: The objective was to assess the impact of synthetic silver nanoparticles (SNPs) on the phylogenetic and functional structure and antibiotic resistome of the microbial community of biofilm-based wastewater treatment system treating municipal wastewater.

Methods: Experiment was performed at biofilm-based wastewater treatment system consisting of six vertical and horizontal flow units treating municipal wastewater. Following treatments were applied: SNPs as collargol with final concentration (as Ag) in inflow 0.1 mgL^{-1}, silver nitrate with final concentration (as Ag) in inflow 0.1 mgL^{-1}, and control. The microbial community was characterized using shotgun metagenomics and quantitative PCR.

Results: The application of SNPs resulted in short-term reduction of wastewater treatment efficiency, especially organic matter removal. The impact of SNPs was more pronounced in vertical flow filter units but the overall system treatment efficiency was compensated by higher removal rate of nitrogen in horizontal subsurface flow units. The analysis of filter units metagenomes indicated the impact of SNPs on biofilm microbial community phylogenetic and functional structure as well as on antibiotic resistome. As a result of the introduction of SNPs, the abundance of all prevalent antibiotic resistance genes (e.g. sul1, sul2, tetA, tetC, tetQ, acrB, catQ, erm(35)) increased in the system outflow compared to the control units.

PW173 Bacterial community structure of two Mediterranean soils amended with spent coffee grounds

María Vela Cano[1], Ana Cervera Mata[2], Jessica Purswani[1], Clementina Pozo[1], Gabriel Delgado Calvo-Flores[2], Jesus Gonzalez Lopez[1]

[1]*Institute of Water Research, Microbiology, Granada, Spain*
[2]*Faculty of Pharmacy, Soil Science and Agricultrual Chemistry, Granada, Spain*

Background: The reuse of spent coffee grounds (SCG) could be a sustainable solution to poor agricultural soils that are often found in Mediterranean regions. However, phytotoxic effects upon direct plantation on soils treated with 2.5% and 10% SCG have been observed. Mainly, phytotoxicity was attributed to the high phenolic acid concentrations in the SCG, hence our aim was to study the effect of SCG concentration on two Mediterranean soils' microbiota (Red and Vega soils).

Objectives: The aim of the current study was to test whether the addition of SCG as a soil conditioner improved the physicochemical properties of the soil and what effect it had on microbial groups.

Methods: Soil microcosms were set up with 2 different soils that were characterized by the "Methods of Soil Analysis". The Folin-Ciocalteu assay was used to measure total phenolic content. DNA samples extracted using the FastDNA® SPIN Kit were analyzed by 454-pyrosequencing.

Results: Results showed a remarkable improvement of physicochemical soil properties (C, N, K) attributed to SCG amendment. Moreover, positive correlations were detected between: phenolic acid degraders and phenolic acid concentrations, as well as plant growth promoting bacteria (PGPB) with time. Microbial interactions increased with SCG amendment, and were highest at 2.5% SCG. Hence, we suggest to allow 30-60 days for SCG mineralization in soil prior to plantation to reduce or eliminate initial phytotoxicity effects and allow for an increase of plant growth promoting.

PW174 The long-term effect of crop rotation and tillage practices on biological soil health indicators

Johan Habig[1;2;3], Johan Labuschagne[4], Mariette Marais[3], Antoinette Swart[3;5], Sarina Claassens[1]

[1]*North-West University, Unit for Environmental Sciences and Management, Potchefstroom, South Africa*
[2]*Agri Technovation, MicroLife Research Centre, Wellington, South Africa*
[3]*Agricultural Research Council, Plant Health and Protection, Pretoria, South Africa*
[4]*Western Cape Department of Agriculture, Directorate of Plant Sciences, Elsenburg, South Africa*
[5]*University of Johannesburg, Department of Zoology, Johannesburg, South Africa*

Background: Agricultural management practices have far-reaching consequences for microbial ecology and soil health. This is especially significant in arid and semi-arid areas such as those found in South Africa. Traditionally, farmers mainly utilised conventional tillage and monocropping in the winter wheat production areas of the country. Gradually, sustainable conservation agriculture approaches are being adopted. This study presents the long-term effects of different agricultural practices by integrating nematode functional guilds with soil microbial diversity and activity data.

Objectives: The objectives were to quantify the temporal impact of cropping sequences and degrees of soil disturbance on (i) nematode trophic levels, (ii) microbial diversity indices and (ii) enzymatic activity in crop production systems in the Western Cape, South Africa.

Methods: During a four-year cropping cycle, the effects of a wheat-medic rotation and wheat monocropping as well as contrasting degrees of soil disturbance (conventional vs zero tillage) were compared. Nematode functional guilds were determined and assigned to a coloniser-persister scale. Microbial species richness and abundance were measured using the Shannon-Weaver and Evenness diversity indices, respectively. Microbial function over time was determined by enzymatic assays (B-glucosidase, phosphatase, urease).

Results: Crop rotation and zero tillage increased nematode trophic linkages, microbial richness and evenness. Plant-feeding nematodes declined over time in conventionally tilled soils. Enzymatic activity related to the carbon, nitrogen and phosphorus cycles increased over time under zero tillage and were independently influenced by cropping sequence. Quantitative analyses of integrated biological indicators under various management practices is important to ensure healthier soil and sustainable crop production.

PW175 Soil bacterial and archaeal community structure and potential of nitrogen cycling processes in well-drained peatland forests

Marika Truu[1], Hiie Nõlvak[1], Kristjan Oopkaup[1], Ivika Ostonen-Märtin[1], Martin Maddison[1], Veiko Uri[2], Ülo Mander[1], Jaak Truu[1]

[1]*University of Tartu, Faculty of Science and Technology, Tartu,*
[2]*Estonian University of Life Sceinces, Institute of Forestry and Rural Engineering, Tartu, Estonia*

Background: In Europe, about 30% of the peatlands have been drained for forestry. Only a limited number of studies have focused on the ecological aspects of soil bacterial and archaeal communities in drained peatland forests.

Objectives: Soil microbial community structure and abundance, as well as genetic potential of nitrogen-cycling processes, was studied in the well-drained peatland forests.

Methods: In total 37 soil samples were obtained from the nine middle-aged forests - three Scots pine forests (*Vaccinium*-type), three Norway spruce forests and Downy birch forests (*Oxalis*-type) located in South-East of Estonia. In addition to the soil microbial characteristics, also soil physicochemical and site-specific plant root parameters, as well as CO_2, N_2O and N_2 fluxes (*in situ* and in laboratory) were measured.

Results: The forest type more strongly affected soil bacterial community structure and abundance than archeal community that proportion was 0.4-6.2% in the total prokaryotic community. The results show that the distance from drainage ditch affected spatial distribution of the forest soil bacterial and archaeal communities but the effect was dependent on the forest type. In most cases, the highest archaeal proportion was measured at the 15m distance from the ditch where also extremely high archaeal *amoA* abundances were measured for two pine forests. Mostly non-linear trends along the distance gradient were revealed for the abundances and proportions of N-cycling genes. The relationships between the structure and abundance of microbial community and plant root parameters and gas fluxes were dependent on the forest type.

PW176 Microbial diversity in Adriatic anchialine caves

Katarina Kajan[1], Neven Cukrov[2], Sandi Orlic[2]

[1]*Institute Ruđer Bošković, Zagreb, Croatia*
[2]*Ruđer Bošković Institute, Zagreb, Croatia*

Background: Anchialine caves can be defined as tidally-influenced subterranean estuary located within crevicular and cavernous karst and volcanic terrains that extends inland to the limit of seawater penetration. The anchialine ecosystem supports a diverse biotic assemblage predominantly comprising stygobiotic species of marine origin, many of which are members of higher taxa that are endemic to anchialine habitats and are distinct from those in contiguous limnic and marine waters. This subterranean estuary is dynamic in that it is affected by marine tides; however, dampened tidal fluctuations and isolation from atmospheric turbulent mixing typically yield sharp physical and chemical stratification between the marine and meteoric waters. The anchialine system merges with the marine system at the coast and fresh groundwater inland. There is very little information on the microbial diversity in these specific ecosystems.

Objectives: The objective of this research is to give a insight on the microbial diversity in the anchialine caves using a sequencing approach.

Methods: Four caves were selected for the analysis at three depths (surface, halocline, saline waters). DNA was extracted and the sequencing of the 16S and 18S were performed at the Illumina platform. Bioinformatic tools were applied for the analysis.

Results: Proteobacteria were the dominant bacterial group in almost all samples, followed by the Firimicutes and Actinobacteria. Alpha, delta and gammaproteobacterial dominated the protebacterial group. Microeukaryotic population is determined by cave and is dominated by Malasseziaceae, Oxyrhinaceceae, Cyclidiidae and Paraphysomonadaceae. A cave specific microbial could be identified in the investigated samples.

PW177 Assessment of Dublin bay water quality: A One Health approach

Laura Sala-Comorera[1], Liam Reynolds[1], Niamh Martin[1], Aurora Gitto[1], Jayne Stephens[1], Tristan Nolan[1], Wim Meijer[1]

[1]*University College Dublin, School of Biomolecular and Biomedical Science, Dublin, Ireland*

Background: Determining the potential impacts of bathing water can be used to design remediation strategies to ensure continued resource use through the challenging period expected from climate change. The improvement of bathing waters is complicated, if a One Health approach is not applied.

Objectives: The aim of this project is to assess the quality of bathing water in Dublin bay and identify the main pollution pressures.

Methods: Deep water samples from the Dublin Bay area were sampled during neap and spring tide and water samples from the seashore were taken every 30 min over a tidal cycle. Faecal indicators (FIs) including somatic coliphages were determined and molecular microbial source tracking analyses were applied.

Results: Analysis of the FIs demonstrated that the samples collected in the deep Dublin Bay area were found to be of excellent quality, however, human marker was identified in 80% of the samples and gull marker in 20%. FIs levels from nearshore water varied one order of magnitude during the tide cycle. These results suggest that bathing water problems originate from nearshore sources such as streams discharging onto the strand, dog fouling and seabirds. Significant differences were found for enterococci and *E. coli* along a day, suggesting a diurnal pattern driven by solar irradiance, therefore the classification of the water quality could be subjected to the sampling time. This study will be of interest to regulatory agencies.

PW178 evaluation of hydrocarbon utilizing bacteria from crude oil -contaminated soil in gio community, niger delta, nigeria

Amara Okoye[1], chioma blaise chikere[2], gideon chijioke okpokwasili[2]

[1]African Centre of Excellence, University Of Port Harcourt, Choba, Port Harcourt, environmental microbiology, choba, port harcourt, rivers state, Nigeria
[2]University of Port Harcourt, , Port Harcourt, Rivers State, Nigeria, microbiology, choba, port harcourt, rivers state, Nigeria

Background: Niger Delta region has suffered decades of untold environmental degradation occasioned by uncontained colossal oil spills.

Objectives:
- To investigate the possibility of existence of viable indigenous hydrocarbon degraders.
- Ascertain amenability of site to bioremediation.

Methods:
- Specific coordinates of the sampling site for the North, South, East and West.
- GCpFID analysis for total petroleum hydrocarbons (TPH) and Polycyclic aromatic hydrocarbons (PAHs)
- Biodegradation screening for the cultivable hydrocarbon utilizing bacteria (HUB)

Results: Longitudes for the specific coordinates were $E7^0$ 13' 49''; $E7^0$ 13'54''; $E7^0$ 13' 53'' and $E7^0$ 13' 54'' for North, South, East and West respectively. The altitudes were 23.87mt (N); 18.66mt (S); 26.47mt (E); 33.64mt (W). Crude oil-polluted samples were collected from the four GPS coordinate points at depth of 0-0.5m (Gio polluted surface soil [GPS]), 1m (Gio polluted sub surface soil [GPSS]) (North, East, West and South) respectively and Gio unpolluted soil (GUPS) to serve as control. GC-FID analysis for TPH were 36,775.65ppm, 14,087.80ppm, 479.67ppm for GPS, GPSS and GUPS respectively. PAHs determined were 12,209.3ppm, 3,248.75ppm, 22.72ppm for GPS, GPSS and GUPS respectively. The pollution level far exceeded Department of Petroleum Resources, intervention limit of 5000mg/kg and 40mg/kg for TPH and PAHs respectively which means the site needs urgent cleanup. *Pseudomonas* spp., *Bacillus* spp., *Achromobacter* sp. *Proteus* sp. and *Serratia* sp. demonstrated high biodegradation of heavy crude oil using 2,6-dichlorophenol indophenols (DCPIP) redox indicator. Conclusions: The extant autochthonous bacteria are metabolically active and could be good candidates for bioremediation of the polluted site.

PW179 Degradation potential of Trametes suaveolens and Trichaptum abietinum in rotating biological contactor in consecutive decolorization cycles

Zuzana Rybková[1], Kateřina Malachová[1], Jakub Hořava[1], Tereza Stachurová[1], Čeněk Novotný[1;2]

[1]*University of Ostrava, Department of Biology and Ecology, Ostrava, Czech Republic*
[2]*Institute of Microbiology ASCR, Laboratory of Environmental Biotechnology, Prague, Czech Republic*

Background: The biological methods employing white-rot fungi can significantly reduce both the color and toxicity of the organopollutants such as synthetic dyes. The use of rotating biological contactor (RBC) for wastewater treatment represents a promising technology for remediation of wastewater contaminated with persistent synthetic dyes.

Objectives: In this study, the performance of a fungal RBC for the degradation of the RBBR synthetic dye was evaluated. The aim was to assess the ability of fungal isolates to degrade the dye in multiple consecutive cycles.

Methods: The strains used were fungal isolates *Trametes suaveolens F1* and *Trichaptum abietinum CA*. RBC reactor consisted of a polymethyl methacrylate vessel and a horizontal driving axis with polyurethane foam disks colonized by the fungus. The experiments comprised six consecutive decolorization cycles. The decolorization of RBBR dye was measured spectrophotometrically and, in parallel, the levels of the extracellular ligninolytic enzymes were measured.

Results: The strains of *T. suaveolens* and *T. abietinum* were able to efficiently degrade the anthraquinone dye RBBR when used in the RBC reactor. *T. suaveolens F1* showed the highest decolorization efficiency of 89 % in 2nd cycle after 12 days. The highest decolorization activity by *T. abietinum CA* was detected in 4th cycle after 8 days, namely 88 %.

Acknowledgements: The work was supported by projects FP-7 KBBE.2012.3.5-02 BIOCLEAN No. 312100 and National Feasibility Program I, project LO1208 TEWEP.

PW180 Microbial composition and contrasts between planktonic and biofouling communities from the Bay of Bengal

Angelina Angelova[1;2], Gregory Ellis[2], Hemantha Wijesekera[2], Gary Vora[2]

[1]*American Society For Engineering Education, Washington, United States*
[2]*U.S. Naval Research Laboratory, Washington, United States*

Background: The BoB undergoes strong influence of many natural and anthropogenic factors, without the overwhelming driving influence of any specific factor. This makes the region uniquely qualified for the exploration of naturally produced planktonic and biofilm microbial communities, typical for a present-day tropical marine environment.

Objectives: This study was aimed at drawing parallels between the composition and variability of planktonic and reciprocal biofilm microbial communities within the Bay of Bengal. Communities were interrogated geographically and vertically within the water column at surface (2m), photic subsurface (75m) and deep-sea (1000m) waters. Consequently, the subsurface planktonic profiles were contrasted against microfouling communities (biofilms), produced onto the surface of reciprocal subsurface moorings. Finally, biofouling community composition was interrogated based on observed biofilm material phenotypes.

Methods: 16S and reciprocal 18S rRNA gene fragments were amplified and sequenced using Ion Torrent S5 platform.

Results: Results indicated vertical stratification of all planktonic communities, with surface and subsurface communities being dominated by anoxygenic and oxygenic photosynthetic organisms (Alphaproteobacteria, Flavobacteria, Cyanobacteria and algae), while the deep-sea communities dominated by sulfur and nitrogen-fixing Archaea and Gammaproteobacteria. Geographic variability was found among the surface and subsurface planktonic communities, while the deep-sea communities remained prevalently stable over the explored range of locations. Defined microbial signatures for each location, however, were not found reflected between reciprocal planktonic and biofilm communities. The observable phenotypes of the biofilm materials was found to reflect its community composition, which in turn, was affected by the integrated macrofouling organisms.

PW181 Culture-dependant and culture-independant evaluation of rhizosphere microbiome of Groundnut (Arachis hypogaea)

Rameshkumar Kothari[1], Ankit Hinsu[1], Prakash Koringa[2]

[1]*Saurashtra University, Department of Biosciences, Rajkot, India*
[2]*Anand Agricultural University, Department of Animal Biotechnology, Anand, India*

Background: Rhizosphere make up the space in the near-vicintiy of roots. Rhizosphere is one of the crucial component of plant development. It has been demonstrated that rhizospheric communities are highly dynamic and changes with changes in the environment factors as well as with crop development and crop rotation.

Objectives: To assess the difference among culture-dependant and culture-independant approaches of microbial diversity evaluation.

Methods: Rhizospheric soil was collected at interval of 2 weeks during nodulation stage of crop. Total of 5 samples were collected during each collection. Isolates were obtained on 8 different media covering broad range of conditions. All the plates were scrapped and DNA was isolated from the mixture. DNA was isolated from all the soil samples using Qiagen DNeasy PowerSoil Kit. 16s rRNA V3-V4 region was amplified and sequenced on Illumina MiSeq using 250bpx2 chemistry. Sequences were compared against greengenes database to assign taxonomy.

Results: As expected, culture-independant approach represented greater diversity compared to culture-dependant approach. However, few genera and species were extensively missed out in culture-independant approach. Notably, *Aeromonas, Klebsiella, Kocuria, Micrococcus, Chryseobacterium, Cronobacter, Curtobacterium* and *Lysinibacillus* were the genera missing in culture-independant approach. This concludes that while culture-independant method is high-throughput for detection of organisms, they tend to fail in identifying some of the organisms. There are chances that left-out organisms might be present in very less abundance as they are not detected during sequencing.

PW182 The impact of combinatorial stress on Burkholderia mesoacidophila is dependent on nutritional conditions but far from predictable

Suzy Clare Moody[1], James C Bull[2], Ed Dudley[2], E Joel Loveridge[2]

[1]*Solent University (Stop IE), United Kingdom*
[2]*Swansea University, Sketty, United Kingdom*

Background: The recently sequenced *Burkholderia mesoacidophila* is a soil organism and as such will be exposed to multiple concurrent stresses in the natural environment. While much has been written about the impact of a single stress, the combination of stresses potentially experienced by microbes in soil has not been investigated in detail.

Objectives: In this study, the impact of a tripartite combination of nutritional availability, temperature challenge and either osmotic or oxidative stress on growth dynamics was investigated. Untargeted metabolomics analysis was conducted to begin to understand the molecular mechanisms of multiple stress tolerance.

Methods: The impact of combinatorial stress on growth was investigated using four different temperatures, two different nutritional conditions, and multiple concentrations of KCl, H_2O_2 or diamide. The differences in growth dynamics were assessed using Generalised Additive Mixed Models (GAMMs) and Nonlinear Mixed-Effects Models (NLMEs). Intracellular metabolite extractions from a subset of samples were analysed by GCMS and compared with the NIST database.

Results: When grown in nutritionally stringent conditions, increasing diamide concentration has no effect on growth while increasing H_2O_2 concentration decreases both growth rate and maximum density. The molecular basis of this difference in response to two types of oxidative stress was investigated and showed clear differences in metabolite profile. The disparity in oxidative stress tolerance was not seen in nutritionally replete cultures. This work demonstrates the specificity of the stress response, and the need to consider multiple environmental factors when investigating tolerance.

PW183 Crop plant and soil pH are strong drivers of rhizosphere microbial diversity in contrast to land use related soil enzyme activities

Claudia Breitkreuz[1], François Buscot[1], Mika Tarkka[1], Thomas Reitz[1], Anna Heintz-Buschart[1]

[1]*Helmholtz Centre for Environmental Research, Department for Soil Ecology, Halle, Germany*

Background: Intensification of land use and on-going alterations in temperatures and precipitation patterns exert an extraordinary pressure on agroecosystems. These drivers curb agricultural productivity and may compromise plant-microbe interactions.

Objectives: Using the research platform "Global Change Experimental Facility", we investigated the impact of these drivers on the structure and function of microbial communities in the rhizospheres of winter wheat and winter barley in two years, 2015 and 2016.

Methods: Rhizosphere samples were taken from conventional (CF) and organic farming (OF) plots of the GCEF, under ambient and future climate treatments. Two developmental stages of each crop, in May and July, were probed. To support the bacterial and fungal community analyses by amplicon sequencing at the functional level, activities of five enzymes involved in C, N and P cycles were investigated.

Results: A strong impact of land use on enzyme activities was detected, in the wheat rhizosphere at both time points and for barley in May samples. Microbial community composition was affected by plant species and soil pH, which varied strongly between plots. The impacts of land use and climate were comparatively weak. Moreover, a poor concordance was detected between measured enzyme activities and PanFP and Tax4Fun predicted enzyme activity potentials of the bacterial communities. Our results indicate that microbial communities with different compositions due to variation in soil pH, may show similar responses to land use and climate change at the level of enzyme activities.

PW184 Modelling the production of extracellular polymeric substances in biofilms

Valentina Gogulancea[1], Rebeca Gonzalez-Cabaleiro[2], Tom Curtis[1], Irina Dana Ofiteru[1]

[1]Newcastle University, School of Engineering, Newcastle upon Tyne, United Kingdom
[2]University of Glasgow, School of Engineering, Infrastructure and Environment, Glasgow, United Kingdom

Background: Extracellular polymeric substances (EPS) are an integral part of biofilm development. They represent complex mixtures of proteins, polysaccharides, lipids and extracellular DNA, essential for biofilm formation. EPS composition varies according to bacterial species and growth conditions, making chemically detailed modelling attempts challenging. At present, most modelling approaches treat EPS as pool of organic carbon, assumed to incur a fixed metabolic cost on the bacterial strain.

Objectives: The aim of this work is to develop a general method to estimate metabolic costs for EPS production, function of chemical composition and environmental conditions, which dictate the free energy available for metabolic processes. EPS production is included in an individual based model to study the relationships emerging between different members of a bacterial community.

Methods: The EPS matrix is assumed to contain only proteins ($C_{16}H_{24}O_8N_5$) and polysaccharides ($C_6H_{10}O_5$) and a generic formula, similar to biomass (e.g. $CH_{1.8}O_{0.5}N_{0.2}$) is proposed by varying the ratios between the two compounds. The EPS free Gibbs energy is computed from the second law of thermodynamics, estimating the entropy and enthalpy values function of the elemental composition. The overall process stoichiometry is obtained by performing a balance between the energy required by the anabolic route (including EPS synthesis) and the one generated by catabolism.

Results: The methodology was tested on bacterial species with known thermodynamic data, showing a satisfactory agreement with experimental values. The influence of protein to polysaccharides ratio on metabolic costs was examined**,** showing higher maximum yields correspond to EPS with lower protein content.

PW185 High-risk clone Escherichia coli O25b-ST131 harboring bla CTXM in healthy cattle from Europe

Josman Palmeira[1;2], Ana Teresa Silva[3], Joana Santos[4], Jean-Yves Madec[5], Marisa Haenni[6], Helena Maria Neto Ferreira[4]

[1]*University of Porto - Faculty of Pharmacy, Porto, Portugal*
[2]*Ucibio*
[3]*University of Porto - Faculty of Pharmacy, Porto, Portugal*
[4]*University of Porto - Faculty of Pharmacy, Porto, Portugal*
[5]*ANSES- Lyon- France*
[6]*ANSES Lyon- France*

Background: Intestinal colonization of food-producing animals with extended-spectrum-beta-lactamase (ESBL) producers represents a public health concern. *Escherichia coli,* an important commensal of the intestinal tract of mammals is responsible for the spread of antimicrobial resistance.

Objective: This study aimed to the characterization of multidrug-resistant (MDR) ESBL-producing *Escherichia coli* in fecal samples of healthy cattle from South of Portugal.

Objectives: Selection of the isolates obtained from fecal samples of cattle, was performed on MacConkey agar with antibiotics, with previous incubation in TSB. Susceptibility-testing was achieved by disk-diffusion-method according to the CLSI. Presumptive identification was performed by CHROMagar-Orientation. ESBL-producers were screened by the double-disk-synergy-test. ESBL-coding genes were screened by PCR. CTX-M-type producing *E. coli* isolates were selected for *Xbal*-PFGE. *E. coli* phylogenetic groups and O25b-ST131 profile were determined by PCR.

Methods: From 117 cattle fecal samples, 460 *Enterobacteriaceae* isolates were obtained, showing a total of 391 MDR isolates, 227 (48,30%) *E. coli* isolates were ESBL-producers (EC-ESBL), from these, 223 were CTX-M-producers. The EC-ESBL-CTX-M gene profile was: $bla_{CTX\text{-}M}$Group1(G1) (87%); $bla_{CTX\text{-}M}$Group9(G9) (12.1%); $bla_{CTX\text{-}M}$G1+$bla_{CTX\text{-}M}$G9 (0.9%). *E. coli* P58 is O25b-ST131, with MDR profile and transfer ability of $bla_{CTX\text{-}M}$G1 by conjugation. PFGE profile was diversified, but showed clonality.

Results: Results show prevalence of $bla_{CTX\text{-}M}$ EC-ESBL (98.24%) presenting a MDR phenotype colonizing these animals. Relevant high-risk clone *E. coli* O25b-ST131 presenting $bla_{CTX\text{-}M}$ was detected. Presence of $bla_{CTX\text{-}M}$-EC-ESBL in animal intestinal colonization shows the animal production as a reservoir of relevant multidrug-resistant-microorganisms, highlighted by the presence of an important human pathogen clone, which is a worrying public health problem.

PW186 Ecophysiological role of an ectophytic bacterium associated with Sphagnum in extreme habitats from Patagonia

Michelle Muñoza[1,2], Michelle Muñoz-Carrasco[2], Mariela Valdésa[2], Gonzalo A. Collado [2], Enrique Werner[2], Rodolfo Caroa[2], Gonzalo Flores-Morales[3]

[1]Universidad de Concepción, Facultad de Agronomía, Chile
[2]Universidad del Bío-Bío, Magister Enseñanza de las Ciencias, Departamento de Ciencias Básicas, Campus Fernando May, Chile
[3]Magister en Ciencias Biológicas, Departamento de Ciencias Básicas, Campus Fernando May, Universidad del Bío-Bío, Chile

Background: Bog mosses of the genus Sphagnum dominate peatlands in Southern South America. In these high latitudes, the bacteria associated with living mosses are not sufficiently understood. These early terrestrial plants were responsible for most of the atmospheric oxygen we have at present and they are considered as the thermoregulator of the planet. Findings could lead to explore interactions between microbial members of the Sphagnum peat community in extreme habitats from Patagonia to give insights into their role in the ecosystem.

Objectives: The present study aims to report the identification of a species of Pseudomonas associated to Sphagnum in Patagonia, Southern Chile and its ecophysiological role related to the microbiome of Sphagnum bogs.

Methods: Sphagnum plants were collected from an ombrotrophic pristine bog close to "Laguna Sin Nombre," a small lagoon located at the Chilean Patagonia. Isolated colonies were grown in LB culture media. DNA isolation and purification was done using Wizard Genomic DNA Kit. Samples were sequenced and a phylogenetic reconstruction was done based on 16S rRNA. The biological activity of the EPS exudate isolated from the bacterium was tested against 4 pathogenic bacteria: Staphylococcus aureus, Listeria monocytogenes, Escherichia coli and Salmonella sp.

Results: Phenotypic characterization and 16S rRNA sequencing data aided to identify the strain as Pseudomonas putida. EPS exudate isolated from the bacterium showed bacteriostatic activity against pathogenic bacteria such as Staphylococcus aureus, Listeria monocytogenes, Escherichia coli and Salmonella sp.

PW187 Culturable extremophile bacteria isolated from modern microbialites from Torres del Paine, Chile

Gonzalo Flores-Morales[1], Patricia Arancibia-Ávila[1], Cristian Torres-Diaz[1]

[1]Universidad del Bío-Bío, Magister en Ciencias Biológicas, Departamento de Ciencias Básicas, Campus Fernando May, Chillán, Chile

Background: Microbialites are organosedimentary deposits formed by the union, precipitation, and capture of sediments product of the growth and metabolism of a benthic microbiome, their presence has been reported in alkaline, saline and oceanic water bodies. They are also considered one of the first known organisms that populated earth and, therefore, their research is imperative since their biogeochemical cycles are representative. In southern Chile, have been no studies concerning the microbiome diversity of microbiolites from Laguna Amarga.

Objectives: The present study aims to report the identification of culturable extremophile bacteria present in modern microbialites.

Methods: A sample of microbialites was collected from Laguna Amarga, a piece was isolated from the sample, which was cultured at 14 °C in LB medium agar, three different colonies were detected. The DNA was extracted using EZNA® DNA Kit. A PCR was carried out using 16S rRNA. PCR products were purified, sequenced and a phylogenetic reconstruction was done. The cultures were analyzed using the API 20/50 kit (bioMérieux® SA).

Results: Three extremophile bacteria were isolated from microbialites present in Laguna Amarga, Torres del Paine, Chile. The analysis of the 16S rRNA gene sequences indicated that the isolated bacteria are highly related to the genus *Halomonas*, *Methylobacterium* and *Dietzia* respectively. Phylogenetic analyses in addition to biochemical tests, indicate that these taxa could be new species. The identification of the cultivable microbiome of the microbialites of Laguna Amarga is of great importance due to its biotechnological and bioremediation potential.

PW188 Exploration of coastal microbial mats for the search of novel oil-degrading microorganisms

Santiago Cadena[1], Daniel Cerqueda Garcia[1], Norberto Ulises García-Cruz[1], Emanuel Hernández-Nuñez[2], Ma. Leopoldina Aguirre-Macedo[1], José Q. García-Maldonado[2]

[1]Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional (CINVESTAV), Unidad Mérida, Recursos del Mar, Mérida, Mexico
[2]CONACYT – CINVESTAV, Mérida, Recursos del Mar, Mérida, Mexico

Background: Marine and coastal environments are often vulnerable to oil contamination. The study of novel hydrocarbonoclastic microorganisms offers a promise strategy for bioremediation of these ecosystems. In this context, photosynthetic microbial mats developing in coastal environments have been largely recognized to harbor a vast phylogenetic and metabolic diversity. However, exploration of hydrocarbonoclastic microorganisms on these habitats has been barely investigated.

Objectives: Here, we evaluate the hydrocarbon-degrading potential by photosynthetic hypersaline microbial mats collected from different coastal environments and characterized the microbial community diversity related.

Methods: Coastal microbial mats were incubated under dark and anoxic conditions with light crude oil for 7 months, in order to obtain an hydrocarbonoclastic microbial consortium. Then, microcosms experiments were conducted to evaluate the oil degradation potential of these culture enrichments and to analyze the microbial diversity associated, using 16S rRNA gene Illumina amplicon sequencing.

Results: An effective hydrocarbonoclastic consortia obtained from microbial mat samples was able to remove light crude oil after 40 days, showing the highest activity within the firs 10 days. High-throughput sequencing analysis showed that the predominant microbial community gradually changed during the biodegradation process. Co-occurrence analysis suggested that most of bacterial taxa were believed to conduct associated interactions for hydrocarbon degradation. We concluded that coastal microbial mats hold a microbial diversity with high biodegradative potential, which is useful for biotechnological purposes.

PW189 A reevaluation of archaeal dissimilatory sulfate reduction

Nikolay Chernyh[1]

[1]*Winogradsky Institute of Microbiology, Federal Research Center of Biotechnology, Moscow, Russian Federation*

Background: Microbial dissimilatory sulfate reduction (MDSR) is an important modern sulfur cycle driver, common in the Bacteria domain, but in Archaea confirmed only for the euryarchaeon *Archaeoglobus*, shown to have acquired the MDSR genes from bacteria. In hyperthermophilic crenarchaea of the family *Thermoproteaceae* the *dsr* genes, responsible for sulfite reduction, are represented by deeply rooted homologues divergent from those of Bacteria and *Archaeoglobus*. However, though claimed for several members, MDSR performed by members of the *Thermoproteaceae* has never been confirmed by presented experimental data.

Objectives: The work aimed to find out if MDSR occurs in terrestrial hot springs favorable for *Thermoproteaceae*, and to identify the agents of the process and the genes involved in “genuinely archaeal" MDSR.

Methods: We used community profiling coupled with ^{35}S-sulfate activity tests, growth and proteomic experiments with "*Candidatus* Vulcanisaeta moutnovskia", isolated from terrestrial thermal acidic environment, as well as such experiments with the type strains of the Thermoproteaceae species previously claimed capable of MDSR.

Results: In acidic hyperthermal springs of the Kamchatka Peninsula, a significant rate of sulfate reduction has been observed and attributed to the *Crenarchaeota* of the genus *Vulcanisaeta*. "*Candidatus* Vulcanisaeta moutnovskia" isolated from these environment was shown to be able to grow by MDSR, while other tested *Thermoproteaceae* lacked this ability. Genomic and phylogenetic studies indicated that in the Crenarchaeota, dissimilatory sulfite reduction may be ancient, and suggested that a later acquisition of qmoABC genes enabled "*Candidatus* V. moutnovskia" to grow by sulfate respiration. This study was supported the RSF grant 17-74-30025

PW190 Intra-species diversity ensures the stability of microbial communities under changing environmental conditions

Natalia García-García[1], Fernando Puente-Sánchez[1], Alexandra Linz[2], Javier Tamames[1], Carlos Pedrós-Alió[1]

[1]*Centro Nacional de Biotecnología (CSIC)*
[2]*Great Lakes Bioenergy Research Center*

Background: Species have been considered the units of microbial diversity. However, both the species concept and its definition are still open issues in microbiology. Likewise, its usefulness as the most significant unit in microbial ecology is also questionable: two individuals classified within the same species may have partially different genomes and carry out different functions in the environment. This intra-species diversity comprises different ecotypes that are assumed to provide stability in time and space to their species.

Objectives: Understanding the role that microdiversity plays as a driver of microbial community stability. This is necessary to predict community response to future disturbances.

Methods: We analyzed 16S rRNA gene amplicons from eight different temperate bog lakes at the OTU-97% and amplicon sequence variant (ASV) levels. We examined whether ASVs behaved as ecotypes through the correlation of their abundance patterns with different environmental variables. We studied the dynamics of ecotypes within the same species across space and time and how their existence affected not only the persistence of species, but also the stability of the whole community itself.

Results: We found ecotypes within the same species with different distribution patterns in space and time. These ecotypes were adapted to different environmental factors such as water temperature and oxygen concentration. Our results showed that the existence of several ASVs within a species favored its persistence and reduced its variability across changing environmental conditions. We propose that microdiversity aids in the stability of microbial communities in the face of fluctuations in environmental factors.

PW191 Combinations of antivirulence compounds and antibiotics are effective against Pseudomonas aeruginosa and can constrain the spread of resistance

Chiara Rezzoagli[1], Martina Archetti[1], Rolf Kümmerli[1]

[1]*Department of Quantitative Biomedicine, Zurich, Switzerland*

Background: Antibiotics are rapidly losing efficacy due to the rapid evolution and spreading of resistance. As an alternative approach to manage infections, treatments targeting bacterial virulence factors have been considered. Antivirulence drugs aim at reducing pathogen virulence instead of viability, and should therefore exert weaker selection for resistance than conventional antibiotics. However, the sometimes low efficacy and the fact that infections are not cleared by the treatment currently compromise clinical applications.

Objectives: Here, we explore the potential of combining antivirulence drugs with antibiotics using *Pseudomonas aeruginosa* as a model system. We hypothesis that such combinations could be beneficial because antibiotics are supposed to increase treatment efficacy, while the antivirulence compound should reduce the spread of resistance.

Methods: We combined two antivirulence compounds: gallium, a siderophore-quencher, and furanone C-30, a quorum sensing-inhibitor, together with four clinically relevant antibiotics. For each drug-pair, we first tested both the efficacy and the nature of interaction using a 9x9 drug concentration matrix. We then competed antibiotic-resistant against susceptible clones under antibiotic and combination treatments.

Results: We found that combination therapy was effective in inhibiting growth and virulence factor production of *P. aeruginosa*. Drug-interaction patterns were concentration dependent, creating a mosaic distribution of antagonistic and synergistic combinations for each drug-pair. Finally, we found that a few drug combinations could indeed prevent the spread of antibiotic resistance. Our work provides a first systematic analysis of antivirulence-antibiotic combinations and suggests that such combinations have good potential to both efficiently treat infections and limit the spread of antibiotic resistance.

PW192 Use of rare-earth elements in the phyllosphere colonizer Methylobacterium extorquens PA1

Lucas Hemmerle[1], Julia Vorholt[1], Ochsner Andrea[1]

[1]*ETH Zürich, Microbiology, Zürich, Switzerland*

Background: Until recently, rare-earth elements (REEs) had been thought to be biologically inactive. This view changed with the discovery of the methanol dehydrogenase XoxF that strictly relies on REEs for its activity. Some methylotrophs only contain *XoxF*, while others, including the model phyllosphere colonizer *Methylobacterium extorquens* PA1, harbor this gene in addition to *mxaFI* encoding Ca^{2+}-dependent enzyme. This raises the question why it harbors both type of enzymes and under which conditions they are expressed.

Objectives: Investigation of the impact of REEs on the expression of *mxaF* and *xoxF* in *Methylobacterium extorquens* PA1.

Methods: Plants were grown under gnotobiotic conditions in a calcined clay based growth system. Mass spectrometry based proteomics was used to measure relative protein abundances across different conditions. ICP-MS was used to determine the concentration of REEs *in planta*.

Results: We showed that *M. extorquens* PA1 is able to grow on methanol in the presence of the REE La^{3+} in the absence of a functional MxaFI enzyme. In presence of La^{3+}, gene expression switched from *mxa* to *xox* in a dose-dependent manner. This implies that the La^{3+}-dependent XoxF is favored over the Ca^{2+}-dependent MxaFI. When PA1 was grown *in planta*, we observed a significant induction of XoxF. Additional proteome investigation on artificial media led to the identification of few proteins that are induced by La^{3+}. One of these was a predicted TonB-dependent receptor. Knock-out mutants revealed its essentiality for growth on La^{3+}, indicating that a dedicated mechanism for REE uptake.

PW193 Assessing Metabolite Biogeography of Rare Actinobacteria Isolated from Marine Environments

Jonathan Parra[1], Katherine Duncan[1]

[1]*Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde, Glasgow, United Kingdom*

Background: The study of biogeography enables an understanding of the distribution patterns of biodiversity across space and time. Therefore, by using a trait-based approach, such as antibiotic production, it is possible to assess the evolutionary, geographic and ecological variables that affect the Actinobacteria specialized metabolism. This is particularly important as Actinobacteria isolated from marine ecosystems have been shown to be a promising source of new drugs.

Objectives: Assess the role of biogeography on the specialized metabolism of *Micrococcus* spp. and *Pseudonocardia* spp. isolated from marine sediments.

Methods: A comparative metabolomics approach, using molecular networking, was applied to analyze the LC-MS/MS profile of phylogenetically related strains isolated from different geographic regions.

Results: Differences in the specialized metabolism of the studied strains were demonstrated. These preliminary results suggest that biogeography influences the microbial chemical space. Future work on further marine ecosystems will expand our knowledge on the relationship between the chemistry and ecology of rare Actinobacteria.

PW194 Methane cycle microbial communities in a meromictic Arctic lake: a winter study

Anna Kallistova[1], Alexander Savvichev[1], Igor Rusanov[1], Vitaly Kadnikov[2], Elena Krasnova[3], Dmitry Voronov[3], Nikolai Ravin[2], Alexander Merkel[1], Nikolai Pimenov[1]

[1]Winogradsky Institute of Microbiology, Research Center of Biotechnology of the Russian Academy of Sciences, Moscow, Russian Federation
[2]Institute of Bioengineering, Research Center of Biotechnology of the Russian Academy of Sciences, Moscow, Russian Federation
[3]Lomonosov Moscow State University, Moscow, Russian Federation

Background: New information concerning activity of methane cycle microorganisms is important for understanding the causes of methane emission from Northern lakes. Despite the fact that Arctic lakes are exposed to a long duration of ice cover, the activity and composition of microbial communities beneath the ice are still poorly understood.

Objectives: To link microbial community profile and methane cycle processes in Arctic Lake Bolshie Khruslomeny (White Sea coast) during the ice-covered season.

Methods: Physicochemical, radiotracer, high-throughput sequencing, and incubation experiments were used.

Results: Pronounced meromixix, presence of the chemo- and halocline, high content of H_2S and CH_4 are unique features of the lake. Lake water is supplemented with sulfate due to contact with the sea basin, and methane cycle is therefore coupled with the sulfur cycle. Aerobic methane oxidation (MO) appeared in the cold-water layer (<1^{o}C) beneath the ice, where psychrotolerant *Methylobacter* predominated; marine methanotrophs were also detected. Anaerobic MO was proposed, but known anaerobic methanotrophs were not found. Diverse methanogens driving hydrogenotrophic, acetoclastic, methylotrophic, and methyl-reducing pathways were identified in both aerobic and anaerobic water layers. The incubations showed that noncompetitive C_1-methylated compounds were most preferable substrates for methanogenesis. Highest potential rate was observed with a mixture of trimethylamine and H_2 as substrate. Isolation of psychroactive methyl-reducing methanogens is currently underway, and improve our understanding of the physiology of these unique microorganisms. Thus, our study might help one insights into the methane cycle microbial communities in Arctic lakes during the ice-covered season.

The work was supported by RSF 16-14-10201.

PW195 Towards a first detailed 16S rDNA-based analysis of the seasonal microplankton dynamics in Lake Constance

Corentin Fournier[1], Anna Kesberg[1], David Schleheck[1]

[1]*Konstanz University, Biology, Konstanz, Germany*

Background: The diversity and annual succession of the zoo- and phytoplankton in Lake Constance is being well-studied as part of a routine sampling program since many years, but the phylogenetic diversity and succession of the microplankton (bacterioplankton) community in this lake is not yet explored in such detail.

Objectives: Here, we present first insight into the diversity and seasonal dynamics of the bacterioplankton in the surface water of the Lake Constance.

Methods: Microplankton, as represented in the size class 5 µm – 0.1 µm, was recovered by filtration and total DNA was extracted. Amplicon sequencing of the V2-V3 region of the 16S rRNA gene was done by Illumina sequencing. Sequences were analyzed using QIIME2 and biostatistics was done using R with the packages Phyloseq and Vegan.

Results: The major groups of bacteria detected throughout all seasons belong to the phyla Actinobacteria, Bacteroidetes, Proteobacteria, Cyanobacteria and Verrucomicrobia, which is typical of freshwater microplankton. These groups are following specific patterns throughout the seasons with a cyclic dynamic within the year. A deeper taxonomic study using co-occurrence analysis and association networks at the OTUs level revealed wide range of intra- and inter-Phyla dependencies. Hence, our study provides a first detailed description of the dynamics and co-occurrence of the bacterioplankton community members at the OTUs level in the surface water of Lake Constance. Its study is continued as part of the DFG Research and Training Group (RTG2272) “R3 - Responses to biotic and abiotic changes, Resilience and Reversibility of Lake ecosystems”.

PW196 Effect of Bacterial Dispersal Networks on Bacterial Activity and Contaminant Degradation in Spatially Disturbed Synthetic Ecosystems

Theodor Mainka[1], Anja Worrich[1], Thomas Banitz[2], Lukas Wick[1]

[1]*Helmholtz-Centre for Environmental Research - UfZ, Environmental Microbiology, Leipzig, Germany*
[2]*Helmholtz Centre for Environmental Research - UFZ, Department of Ecological Modelling, Leipzig, Germany*

Background: Microbes in soil are often exposed to environmental disturbances. However, little is known on how the and spatial structure of disturbances influences the microbial turnover of anthropogenic contaminants. Previous *in silico* studies suggest that especially spatially clumped disturbances reduce the biodegradation function and that bacterial motility and dispersal along preferential networks are essential for functional recovery from such disturbances.

Objectives: Therefore, we assessed the impact of spatially confined disturbances on the functional stability of bacterial contaminant degradation in synthetic ecosystems with and without bacterial dispersal networks.

Methods: We developed an agar-based synthetic ecosystem using the motile strain *Pseudomonas putida* KT2440-gfp and its non-motile mutant KT2440-Δflim dsRed. Recurrent and spatially confined disturbances were applied via UV-light. Glass fibers served as preferential networks for bacterial dispersal, thus mimicking effects of fungal hyphae in natural ecosystems. We assessed the bacterial abundance and recolonization of disturbed areas by cell counting and microscopy tools. The bacterial functional performance was measured in terms of benzoate degradation.

Results: The bacterial recolonization of disturbed areas was fast when disturbances occurred in a spatially fragmented pattern, but slow after spatially clumped disturbances. Dispersal networks helped bacteria to recolonize these clumped disturbed areas and, thus, improved functional stability. The results indicate that spatial structure and microbial dispersal ability are decisive for the extent and stability of microbial contaminant degradation in disturbed environments.

PW197 Overall transition patterns and the compositions of microbial modules during succession of Microcystis genotypes using network analysis

Seong-Jun Chun[1;2], Yingshun Cui[2], Jay Jung Lee[3], Hee-Mock Oh[1;2], Chi-Yong Ahn[1;2]

[1]Korea University of Science and Technology (UST), Department of Environmental Biotechnology, KRIBB School of Biotechnology, Daejeon, Korea, Rep. of South
[2]Korea Research Institute of Bioscience and Biotechnology (KRIBB), Cell Factory Research Center, Daejeon, Korea, Rep. of South
[3]National Institute of Environmental Research, Geum River Environment Research Center, Okchun, Korea, Rep. of South

Background: *Microcystis*, major bloom forming cyanobacterium, interact with variety of microbes and their association networks have been revealed by recent studies, yet the ecological relationships between genotypes of *Microcystis* and microbes are largely unknown.

Objectives: Here, we investigated the dynamics of cyanobacteria, including genotypes of *Microcystis*, heterotrophic bacteria, and eukaryotes to elucidate the ecological connections among primary producers, consumers, and decomposers during cyanobacterial harmful algal blooms (cyanoHABs).

Methods: Freshwater samples were collected weekly from June 2017 to September 2017 at surface from three sites on Daechung reservoir, Korea. we investigated diversity and compositions of cyanobacterial, including genotypes of *Microcystis*, heterotrophic bacterial, and eukaryotic communities using high-throughput sequencing. We constructed microbial network and identified their distinct modular structure.

Results: Network analysis revealed that the overall transition patterns and the compositions of modules (microbial clusters) that involved in the same phase of cyanoHABs showed resemblance in three different sites. Distinct clusters of *Microcystis* genotypes and microbes were observed in different types of *Microcystis* bloom, suggesting the alteration of *Microcystis* genotypes could mediate by their specific companions as well as environmental factors. Most of *Microcystis*-related microbes (16S rRNA based) were also directly linked to different genotypes of *Microcystis*. In addition, hidden members of bloom modules were also tightly coupled with genotypes of *Microcystis* and may support communities function profiles during *Microcystis* bloom periods. Therefore, to understand complex ecological interactions during *Microcystis* blooms, we should consider their interactions through a network and modular structures based on the *Microcystis* genotypes.

PW198 Single-cell metabolic heterogeneity in monoclonal microbial populations is shaped by abiotic factors

Federica Calabrese[1], Hryhoriy Stryhanyuk[1], Florin Musat[1], Hans Hermann Richnow[1], Martin Thullner[2], Lukas Wick Gessler[2], Niculina Musat[1]

[1]*Helmholtz Center for Environmental Research, Isotope biogeochemistry, Leipzig, Germany*
[2]*Helmholtz Center for Environmental Research, Environmental microbiology, Leipzig, Germany*

Background: Populations of genetically identical microorganisms residing in the same environment can display marked variability in their metabolic traits. The relevance of such heterogeneity in natural habitats and the main factors that trigger metabolic heterogeneity at single cell-level are largely unknown. This is mostly because measurements of a statistically relevant number of single-cell metabolic activities of the same species in complex microbial communities are technically difficult and were not even possible until few years ago.

Objectives: In this study we aimed to understand if abiotic factors i.e. electron donors, also used as carbon sources, and electron acceptors can cause metabolic heterogeneity in putative monoclonal bacterial populations and if such heterogeneity shapes the species degradation capabilities**.**

Methods: As model organisms, we used Pseudomonas putida KT2440, Pseudomonas stutzeri and Thauera aromatica, ubiquitous in terrestrial environments and known for their capability to degrade aromatic compounds. Batch cultures were grown with various concentrations of 13C-labelled acetate or benzoate under oxic and anoxic conditions. Cells were sampled during their exponential growth phase and single cells 13C uptake was investigated thanks to the combination of stable isotope probing and nanoscale Secondary Ion Mass Spectrometry (nanoSIMS).

Results: Our preliminary data shows that intra-population heterogeneity is strain dependent and strongly influenced by electron donor concentration. These results have implications in understanding the ecological and functional role of metabolic heterogeneity and its potential impact on pollutant´s biodegradation in the environment.

PW199 Effects of 17-b-estradiol and diclofenac on growth and chlorophyll content of Chlorella vulgaris

Paula Coutinho[1], Andreia Alves[1], Inês Martins[1], Maximiano P. Ribeiro[1], Márcio José de Abreu Marques Rodrigues[1], Andre R.T.S. Araujo[1]

[1]*Polytechnic institute of Guarda, Guarda,*

Background: In the last years European Union defined a Watch List of aquatic contaminants of emerging concern, including estrogens, pharmaceuticals, pesticides. Their presence in the environment may cause undesirable effects on ecosystems, namely endocrine disruption as also the development of resistant bacteria.

Objectives: The primary aim of this study was to assess the toxicity of 17-β-estradiol and the non-steroidal anti-inflammatory drug diclofenac in the microalga *Chlorella vulgaris*, as an organism model for ecotoxicological assays.

Methods: The growth inhibition test of compounds in *C. vulgaris* was carried out according to OECD guidelines (OECD, 2011) for 24, 48 and 72 h in photobioreactors with 80 mL of culture, containing a series of drugs concentration considering the range described in the literature for its presence in natural aquatic environments.

Results: Results indicated that the growth of microalga and the content of chlorophyll were significantly affected by tested contaminants. *C. vulgaris* presents bioremediation capacity, once the drug's concentration during the assay (24, 48 and 72 h) decrease, even considering equally the degradation under irradiance that seems to be specially relevant for 17-β-estradiol.

PW200 Mathematical modelling of plasmid ecology and evolution

Jamie Wood[1]

[1]*University of York, Mathematic and Biology*

Background: Plasmids are a key vector for horizontal gene transfer in bacteria, providing a rapid mechanism for the transfer of genes between bacteria of both related and unrelated species. Genes on plasmids provide a wide range of environmentally and clinically relevant traits, including metabolic genes, antibiotic resistance genes and virulence factors. The ecology of the plasmids themselves is also of fundamental scientific importance, with complex cost/benefit ratios determining fitness and hence the evolutionary fate of both host and plasmid.

Objectives: Here I present an ongoing journey of a mathematical modeler into the world of plasmids and their awkward mathematical position on a timescale that sits between ecology and evolution.

Methods: I will present work looking at the ecology of the retention and segregative loss of plasmids in response to toxic selective pressure, phage selective pressure and the presence of communities both empirically and mathematically.

Results: I will discuss the evolution of both host and plasmid in response to the presence of antibiotics, and the emergence of intra-genomic co-evolution between plasmid and host. Finally, I will introduce a more recent project and the mathematical challenges present by considering how the interaction of ecology and evolution determines the fate of the host-plasmid system under selection.

PW201 Pseudomonas aeruginosa mobbs protozoan predators

Nimrod Shteindel[1], Yoram Gerchman[2]

[1]*University of Haifa, Evolutionary and Environmental Biology, Haifa, Israel*
[2]*Haifa university at the Oranim Academic College campus, Biology, Kiryat Tiv'on, Israel*

Background: Mobbing, a collaborative attack on a predator by prey organisms, is reported in many animal species, but not in bacteria. *Pseudomonas aeruginosa*, a common opportunistic human pathogen is known to kill predatory protozoa, but this phenomenon was previously discussed only in the context of the molecular mechanisms facilitating it.

Objectives: We look for mobbing behavior in *P. aeruginosa*, testing effects of Quorum sensing (QS) and nitric oxide (NO), hypothesized to be used by bacteria as measures for conspecific and predator population density respectively.

Methods: We used a kinetic mesurment in a high throughput setting, to measure attachment in *P. Aeruginosa* PAO1 WT and ΔPqsA (QS deficient mutant), in the presence of of *Acanthamoeba castelanii, Paramecium tetraurelia* in several predator population densities. QS mutant was complemented with various concentration of the missing QS inducer. Paramecia were treated with NO synthase inhibitor to investigate the idea that NO is used as a predator kyromone by the bacteria.

Results: *P. aeruginosa* attachment is seen within seconds, in positive corelation to amoeba population density (surface dwelling) and negative to paramecium (free swimming, when attaching to a paramecium, bacteria do not attach to plastic surface). Microscopy shows *P. aeruginosa* attaches to protozoa within seconds. QS mutant shows reduced attachment in the presence of amoeba, reverting when QS inducer is added. NO synthase inhibition in paramecia reduces the effect by half. These findings suggest mobbing behavior in *P. aeruginosa*, effected by predator and prey population density, precived using NO and QS.

PW202 Calibration of 16S rRNA gene based microbial community results bias using internal standards

Changwoo Park[1], Seil Kim[1]

[1]KRISS

Background: The microbiological community analysis is can be used to find out beneficial and harmful microorganisms in various environments including food, human body and etc. Especially, the microbial community structures can be illuminated by the genetic analysis based on the 16S rRNA genes. Recently, Next-Generation Sequencing (NGS) technologies enabled rapid, parallel and accurate microbial community analysis of hundreds of samples from various environments. However, there are various sequencing platforms and the microbial profile from different platforms can show different results

Objectives: The calbriation of microbial community analysis results based on 16S rRNA using Internal standard (IS)

Methods: Nineteen probiotics strains were selected and corresponding internal standards material (IS) were synthesized. The IS and corresponding 16S rRNA genes were amplified and sequecned by various universal primer pairs and d various seqeuncing plaforms. The processing and analysis of raw sequences was done using MOTHUR.

Results: The microbiological profile results of the platform were calibrated by internal standard materials. The corrected microbiological profile results can be improved by reducing the bias from sequencing platform and primer pairs. It indicated that the internal standard material can be used to provide compatibility with the microbial community analysis results.

PW203 Single-cell trait-based biodiversity in microbial communities and its link to eco-system functioning in a stratified lake

Simone Fontana[1], Stephane Escrig[2], Francesco Danza[3], Anders Meibom[2], Francesco Pomati[1], Martin Ackermann[1;4], Frank Schreiber[1;4;5]

[1]*Eawag, Swiss Federal Institute for Aquatic Science and Technology*
[2]*École Polytechnique Fédérale de Lausanne (EPFL)*
[3]*Supsi, Laboratory of Applied Microbiology*
[4]*ETH Zurich*
[5]*Federal Institute for Materials Research and Testing (BAM), Department of Materials and Environment*

Background: A fundamental question in ecology is how biodiversity affects ecosystem function. Biodiversity is commonly estimated based on genetic variation.

Objectives: We investigated a new approach that defines and measures biodiversity in complex microbial communities. We used the variation in multiple functionally-relevant, phenotypic traits measured in parallel in single cells as a metric for microbial phenotypic diversity.

Methods: We studied phenotypic diversity and ecosystem functioning in photosynthetic layers dominated by divergent microbial communities in the gradient of Lago di Cadagno. We determined genetic diversity by 16S and 18S amplicon sequencing and bulk ecosystem functioning (photosynthesis). In addition, we determined phenotypic diversity using single-cell technologies such as nanometer-scale secondary ion mass spectrometry (NanoSIMS) correlated with confocal laser scanning microscopy and scanning flow-cytometry. We measured functional trait variation between individuals in 13CO2 fixation, 15NH4+ uptake, and variation in physio-morphological cell traits, such as cell size, shape, and auto-fluorescence for various pigments related to photosynthesis. We used the distances between individuals in a multidimensional trait space to derive phenotypic trait-based diversity indices, such as trait richness, trait evenness, and trait divergence.

Results: We find that phenotypic trait divergence associates with ecosystem functioning, whereas genetic diversity does not. Including activity-based, single-cell phenotypic measurements with NanoSIMS provided an additional accuracy to the trait-based diversity assessment and allowed us to formulate hypotheses on the mechanisms that shape the correlation between phenotypic diversity and eco-system function. Together, our results show that phenotypic diversity is a meaningful concept to measure microbial biodiversity and associate it with ecosystem functioning.

PW204 Soil physico-chemical characteristics drive fluorescent Pseudomonas and cyclic lipopeptide diversity in the rhizosphere of cocoyam

Feyisara Eyiwumi Oni[1], Niels Geudens[2], Olumide Owolabi Omoboye[1], Anthony Argüelles Arias[3], Joseph Onyeka[4], Oluwatoyin Faith Olorunleke[1], Ayodeji Salami[5], Amayana Adiobo[6], Stefaan De Neve[7], Marc Ongena[3], José Martins[2], Monica Höfte[1]

[1]Ghent University, Laboratory of Phytopathology, Department of Plants and Crops, Ghent, Belgium
[2]Ghent University, NMR and Structural Analysis Unit, Department of Organic and Macromolecular Chemistry, Faculty of Science, Ghent, Belgium
[3]University of Liege, Faculty of Gembloux Agro-Bio Tech, Microbial Processes and Interactions Unit, Gembloux, Belgium
[4]National Root Crops Research Institute (NRCRI), Plant Pathology Unit, Umudike, Nigeria
[5]Ekiti State University, Faculty of Agriculture, Ado-Ekiti, Nigeria
[6]Institute for Agricultural Research for Development (IRAD), Jay P Biotechnology Laboratoty, Cameroon
[7]Ghent University, Research Group of Soil Fertility and Nutrient Management, Department of Soil Management, Ghent, Belgium

Background: Soil microbial diversity has long been considered as a key indicator of soil quality and health. In a previous study, we showed that the rhizosphere of the tropical cocoyam crop in Cameroon is rich in fluorescent pseudomonas taxonomic diversity and also a hotspot for cyclic lipopeptide (CLP) metabolites. However, factors contributing to this striking diversity are unclear.

Objectives: This study aimed to elucidate the influence of soil characteristics on *Pseudomonas* taxonomic and CLP diversity in the cocoyam rhizosphere.

Methods: Thus, we collected *Pseudomonas* isolates from five different fields situated in Cameroon and Nigeria. Taxonomic characterization was done using the *rpoD* housekeeping gene while the presence of CLP production and types were deciphered using the drop collapse test and UPLC-MS, respectively.

Results: We obtained 138 *Pseudomonas* isolates from andosols in Cameroon (Boteva, Ekona and Maumu villages) and 165 from alfisols and ultisols in Nigeria (Ado-Ekiti and Umudike). Physico-chemical analyses showed that compared to other soils, soils from Boteva recorded higher organic carbon, N, Ca and silt. Furthermore, 50% of isolates obtained from Boteva belonged to the *P. fluorescens* complex while the rest were *P. putida* group isolates. In contrast, other locations mainly recorded the presence of *P. putida* isolates. For CLP types, *Pseudomonas* isolates from Boteva produced 10 different CLPs including four novel ones while those from Ekona, Maumu, Ado-Ekiti and Umudike produce five, five, three and two CLPs, respectively. Our study suggests that CLP diversity and presence of *P. fluorescens* complex isolates are indicators of good soil quality.

PW205 Bacillus subtilis practises promiscuous but safe sex

Polonca Stefanic[1], Katarina Belcijan[1], Barbara Kraigher[1], Rok Kostanjsek[2], Ines Mandic-Mulec[1]

[1]*University of Ljubljana, Biotechnical Faculty, Department of Food Science and Technology, Ljubljana, Slovenia*
[2]*University of Ljubljana, Biotechnical Faculty, Department of Biology, Ljubljana, Slovenia*

Background: *B. subtilis* is a soil dwelling bacterium with a diverse social life that includes interactions resulting in bacterial equivalent of sex, which subsequently have a profound influence on bacterial evolution. Recently we discovered kin discrimination among highly related strains of *B. subtilis*, where less related strains showed antagonistic behaviour towards each other in the form of killing, however, the role of kin discrimination in bacterial sexuality remains unknown.

Objectives: Our aim was to identify how kin discrimination affects horizontal gene transfer and to find mechanisms responsible for gene exchange.

Methods: Swarming assays were performed and samples were taken from the meeting points of kin and non-kin swarms. DNA concentration, DNA exchange and activation of competence genes was determined by QuantiFluor® system, selective plating and fluorescent microscopy, respectively. Cell integrity was investigated by electron microscopy.

Results: We show that strains of *B. subtilis* preferably take up DNA from less related non-kin strains and this mechanism stems from a higher induction of competence at the meeting point of two non-kin strains due to cell-envelope stress response. Our results demonstrate an important evolutionary mechanism of ''promiscuous but safe sex'': a type of bacterial cell-contact dependent DNA exchange that could promote diversification inside the species but still bring low risk of integrating toxic DNA. Our findings help understand the vast genomic diversity within this species despite existing mechanisms limiting less-related DNA integration during transformation.

PW206 Cochlodinium polykrikoides bloom is associated with the distinct bacteria, archaea, and phytoplankton clusters in South Sea of Korea

Yingshun Cui[1], Seong-Jun Chun[1], Seung Ho Baek[2], Pyoung-Joong Kim[3], Moonho Son[3], Hee-Mock Oh[1], Chi-Yong Ahn[1]

[1]Korea Research Institute of Bioscience and Biotechnology, Cell Factory Research Center, Daejeon , Korea, Rep. of South
[2]Korea Institute of Ocean Science and Technology
[3]National Institute of Fisheries Science

Background: Harmful algal blooms (HABs) of the *Cochlodinium polykrikoides* cause huge economic and ecological damages, thus are of environmental problems in Korea. Previous studies uncovered that the formation and collapse of phytoplankton blooms could be closely related to their associated microbes, while almost no reports to elucidate the roles of microbes in *C. polykrikoides* blooms in field.

Objectives: To explore the potential links between *C. polykrikoides* and other microbes, we collected water samples in different size-fractions when *C. polykrikoides* blooms were occurred in South Sea of Korea.

Methods: Bacteria, archaea, and phytoplankton community structures were deep-sequenced using next-generation sequencing approach.

Results: Compared with the non-bloom samples, Marine group I (archaea), *Micrococaceae* and *Piscirickettsiaceae* (bacteria), and *Syndiniales* group I (phytoplankton) were more enriched in *C. polykrikoides* bloom period. Network analysis showed that *C. polykrikoides* module compositions significantly differed from those of other modules that included *Alexandrium*, *Chaetoceros* or *Chattonella*, indicating specific microbial clusters were related to *C. polykrikoides* bloom. Among the OTUs directly correlated with *C. polykrikoides*, Marine group I could supply vitamin B_{12}, the essential element for *C. polykrikoides* growth, while the potential fish pathogens (*Micrococaceae* and *Piscirickettsiaceae*) could contribute to massive fish death together with *C. polykrikoides* itself. In addition, the zoospore of *Syndiniales* might be related to the sudden collapse of *C. polykrikoides* blooms. Collectively, our results showed new insight into the mechanism of rise and fall in *C. polykrikoides* blooms in the aspect of microbial interaction.

PW207 phenotypic, biochemical and genomic characterization of the cypermethrin degrading and biosurfactant producing bacterial strains isolated from the northern chilean patagonia

Patricia Aguila[1], Mauricio Gonzalez[2], Jonathan Maldonado[3], Alexis Gaete[3]

[1]*Universidad Austral de Chile, Escuela de Tecnologia Medica, Puerto Montt, Chile*
[2]*INTA-Universidad de Chile, INTA, Santiago, Chile*
[3]*INTA-Universidad de Chile, Santiago, Chile*

Background: The pesticide causes a severe environmental damage on marine ecosystems. Cypermethrin is an antiparasitic pesticide used extensively in the aquaculture industry.

Objectives: The objective of this study was the isolation and characterization of the cypermethrin-degrading and biosurfactant-producing bacterial strains.

Methods: Thirteen marine bacteria were isolated by enrichment from a cypermethrin contaminated sediment samples at southern Chile. Isolates were identified by 16S rDNA genes sequenced analyses. Isolates were tested for biosurfactant production and emulsification activity.

Results: Marine strains belonged to four different genera, including *Pseudomonas*, *Rhodococcus, Serratia* and *Moraxella*. All isolates were capable to grow on cypermethrin, however, four of them reaching highest growth on cypermethrin and these in turn synthesized biosurfactants based its high emulsification index E_{24}. This was the case of MS13 and MS16 in case of *Rhodococcus* genera and MS15 and MS19 in case of *Pseudomonas* genera. These strains were genetically, biochemically, and morphologically characterized. Esterase genes *in silico* related to biodegradation of cypermethrin were identified. These novel cypermethrin-degrading and biosurfactant-producing bacterial strains have a biotechnological potential for bioremediation of cypermethrin contaminated marine sediments.

PW208 Long-term monitoring of indoor air microbiome in dwellings: can we predict asthma exacerbation ?

Louise-Eva Vandenborght[1;2], Coron Noémie[2;3], Raphael Enaud[2;4], Charlotte Urien[1], Pierre-Olivier Girodet[2], Patrick Berger[2], Stephanie Ferreira[1], Laurence Delhaes[2;3]

[1]*GenoScreen, Research and Development, Lille, France*
[2]*University of Bordeaux, INSERM U1045 Cardiothoracic Research Center, UMR 1045, Bordeaux, France*
[3]*Hospital of Bordeaux, Mycology and parasitology, Bordeaux,*
[4]*Hospital of Bordeaux, CRCM Pediatry CIC 1401, Bordeaux, France*

Background: The link between fungi exposure and asthma is nowadays well-admitted but few studies has combined results from pulmonary and indoor air microbiome of asthmatic patients in regards to exacerbation risk.

Objectives: We therefore investigate the connection between indoor and pulmonary microbiome in order to demonstrate that a long-term monitoring of indoor air mycobiome could be a tool to prevent acute exacerbations of asthma.

Methods: The indoor air microbiome of 49 asthmatic patients from the COBRA cohort have been recovered with Electrostatic Dust Collectors during 10 weeks. In parallel, we collected sputa of 25 of these patients during stable and/or pulmonary exacerbation periods. We used targeted metagenomic methodology to characterize microbial communities from all samples with the Illumina Miseq platform.

Results: Most of the identified fungi belong to Ascomycota (>50%). However, the Basidiomycota phylum is more represented in indoor air (40.8%) compared to sputa (21.4%). At the genus level, we mostly identified *Cladosporium*, *Hyphodontia*, *Malassezia*, *Aspergillus* and *Penicillium* in indoor air. These last three being also the main genera found in sputa, it suggests a possible link between endogenous and exogenous microbiota. Further investigations are now under way to confirm this link. Additionally, differential abundance analyses of the fungal communities in sputa according to the clinical status highlighted a significant increase in the exacerbated group, of not only the well-described *Aspergillus fumigatus* but also several others fungi. This could suggest that a more complex fungal community than yet described could be implicated in a negative clinical evolution of asthmatic patients.

PW209 Methanogenesis in a temperate estuary

Lynsay Blake[1], Angela Sherry[2], Neil Gray[2], Ian Head[2]

[1]*Durham University, Durham, United Kingdom*
[2]*Newcastle University, United Kingdom*

Background: In the face of global challenges such as climate change, changing land use, and extensive urbanization there are increasing calls for 'coordinated, cross-disciplinary efforts…to understand, predict, and harness microbiome function'. Before we can harness the full potential of the global microbiome we have to understand the range of function it can offer. Exploration of extreme environments has been pivotal in uncovering microorganisms with exploitable function. Fewer studies have focused on understanding the full microbial potential of more pedestrian environments.

Objectives: Methanogenic potential of sediments from a temperate estuary were investigated in response to substrate, temperature, and salinity amendment. The hypothesis being that because estuaries act as conduits between freshwater and marine environments, they will display a broad range of methanogenic function.

Methods: Anoxic sediment microcosms were amended with methanogenic substrate incubated at temperatures from 5 to 70°C, and NaCl 0 - 137gL^{-1}. Headspace methane was measured by GC-FID. Microcosms were sacrificed for community analysis.

Results: Methanogenic thermal range was broad (5 – 70 °C; with bimodal T_{opt} at 30, and 60-70 °C). At 30°C, methanogenic substrate amendment enriched for different trophic groups, but, at 60°C only hydrogenotrophs i.e. genus *Methanothermobacter* were enriched. The salinity tolerance of mesophilic hydrogenotrophic and methylotrophic methanogens (30°C) was high (≤137gL^{-1} NaCl). In contrast, the activities of estuarine thermophilic methanogens (60°C) were limited to <50 gL^{-1} NaCl, regardless of substrate addition. Globally, individual cultured methanogen temperature and salinity characteristics map closely to their reported isolation sources ranging from arctic to hydrothermal and freshwater to hypersaline settings.

PW210 Characterization of microbial communities hosted in watersheds of Tatun volcano group, Taiwan

Bing-Mu Hsu[1]

[1]*National Chung Cheng University, Earth and environmental sciences, MinXiong, Taiwan*

Background: The geochemical studies revealed that the relatively high concentrations of sulfur and arsenic and the more presenting heavy metal iron like lead were found in the Tatun Volcano Group and its adjacent watersheds. It is a useful strategy to deal with the pollution of environmental heavy metal by studying the investigation of the interactions between microbial communities and environmental chemicals intensively.

Objectives: To understand the characterization of microbial communities hosted in watersheds of Tatun Volcano Group, Taiwan.

Methods: The aquatic samples and biofilms were collected from Huang-gang creek and Beitou creek for investigating the microbial communities and quantitative evaluation of related functional genes. Bacterial 16S rRNA of V3-V4 gene region of the total samples were amplified and identified by next-generation sequencing.

Results: The dominant Phyla of samples from Huang-gang creek were Cyanobacteria/Chloroplast, Firmicutes and Proteobacteria and a unique group consisting of environmentally tolerant bacteria and thermophilic acidophilus were found in the geothermal valley and the downstream of the Huang-gang creek. Based on the study of the bacterial community, the heavy metal resistance potential of each sample could be evaluated by the KEGG database. The outcomes indicated that the resistance gene expression of arsenic, iron, and sulfur from the Huang-gang Creek stream samples are presenting relatively high in the water phase, while the lead resistance gene expression is mainly manifested in the biofilms.

PW211 Of Microscopes and Microbes: novel applications of advanced optical microscopy for microbiology

Liam Rooney[1], Paul Hoskisson[1], Gail McConnell[2]

[1]*University of Strathclyde, Strathclyde Institute of Pharmacy and Biomedical Sciences, Glasgow, United Kingdom*
[2]*Department of Physics, University of Strathclyde, Glasgow, United Kingdom*

Background: Optical microscopy development has been traditionally driven by the needs of mammalian cell biology. Therefore, microbial imaging has been somewhat overlooked. To address this, we have applied several advanced optical microscopy techniques to probe the architecture of *Escherichia coli* biofilms and understand the gliding behaviour *Myxococcus xanthus*.

Objectives: Our main objective was to study bacterial community dynamics using novel applications of advanced optical microscopy techniques.

Methods: We used the Mesolens to visualise the architecture of *E. coli* colony biofilms. The Mesolens is a large optical microscope with the unique combination of a low magnification and a high numerical aperture. This results in an imaging volume of >100 mm^3, with 700 nm lateral and 7 μm axial resolution throughout. We also used interference reflection microscopy (IRM) to study the gliding dynamics of *M. xanthus*. This label-free super-resolution technique relies on detecting reflected incident light from refractive index boundaries in the sample, where changes of <100 nm can be detected in the axial position of live cells.

Results: Using the Mesolens we have characterised a previously undocumented network of intra-biofilm channels which play a role in the uptake of nutrients and other substances from the external environment. We have demonstrated the ability to transport fluorescent microspheres and small nutrients through these structures using a fluorescent biosensor. Using IRM we show novel insights into the gliding behaviour of *M. xanthus* where motile cells lift regions of their cell body as an inherent part of their gliding motility behaviour.

PW212 Multi-scale variability analysis of Arctic soil microbial communities

Lucie Malard[1], David Pearce[1]

[1]*Faculty of Health and Life Sciences, Northumbria University, Newcastle Upon Tyne, United Kingdom*

Background: Understanding distribution patterns at various spatial scales is a central issue in microbial ecology. Beyond the lone identification of biogeographical patterns, understanding the environmental drivers behind community diversity and structure is key. While many studies identify pH as a major parameter structuring microbial communities at large spatial scales, many other variables impact distribution patterns on smaller scales.

Objectives: We investigated the spatial distribution of Arctic soil microbial communities from 1 m to 500 m, within Adventdalen, Svalbard. We also identified key environmental factors and chemical elements influencing bacterial assemblages.

Methods: Using high-throughput 16S sequencing, gravimetric measurements and X-ray fluorescence, we characterized microbial communities and soil physico-chemical properties. Using multivariate analyses and geostatistics, we identified key environmental factors and mapped bacteria distribution across the landscape.

Results: Multivariate analyses identified key environmental variables shaping microbial communities and revealed the importance of soil moisture, organic carbon and elements such as aluminum, calcium and potassium in structuring distribution patterns. The indicator species analyses identified key associations between environmental variables and OTUs. Using geostatistical kriging, we mapped the biodiversity and distribution of key OTUs across the landscape. Overall, our results highlight the spatial heterogeneity in Arctic soils and identifies the sampling scale needed to characterize microbial communities within an area of interest with seemingly homogeneous landscapes.

PW213 Microbial Degradation of Industrial effluents by a Novel Tannase Enzymes

Sarita A. Bhutada[1]

Department of Microbiology, Sanjivani Arts, Commerce and Science College, Kopargaon, (INDIA)

Backgrounds: Tannery effluents containing large amount of wastes especially tannins when discharged into watercourse affects physical, chemical and biological characteristics of water and deplete the dissolved oxygen, which in turn affects the aquatic life.

Objectives: The aim of this study was to isolate a tannase producing fungi from soil and to study biodegradation of industrial effluents by tannase enzyme.

Method: In the present, tannery effluent wastes were collected from the industrial area and are ranked as one of the high polluting wastes due to a heavy load of pollutants. The collected tannery effluent showed very high BOD and COD values. Tannase producing fungi were isolated using the simple agar plate method followed by the screening of organisms capable of producing tannase using the enrichment culture technique in modified Czapek Dox's agar. Finally, tannase obtained from the best isolate was partially purified and characterized.

Results: An indigenous fungal isolate from soil near the effluent discharged area with potential tannase activity was selected for degradation study and was identified as Aspergillus spp. Some fungal strains namely *Aspergillus niger*, *Aspergillus rugulosa*, and *Aspergillus fumigatus* were isolated, characterized morphologically and by their capacity to degrade tannins. Aspergillus showed the highest tannin degrading capacity. Biodegradation of tannery effluent by *Aspergillus* spp. decreases its tannin content and represents a valuable source of tannase for potential application in various industries.

PW214 Temporal Variability of the Microbial Ecology in the Coastal Waters of Pensacola Beach, Florida

Arianna Simmering[1], LIsa Nigro[1], Erika Neat[2], Melissa Ederington-Hagy[2], Melissa Brock[1], Wade Jeffrey[1]

[1]University of West Florida, Center for Environmental Diagnostics and Bioremediation, Pensacola, United States
[1]University of West Florida, Center for Environmental Diagnostics and Bioremediation, Pensacola, United States

Background: Microbial communities are greatly influenced by environmental factors as well as disturbance events. Of equal interest is their resiliency and recovery from those disturbances.

Objectives: Our goal was to investigate changes in microbial communities over different time scales. These include a three year seasonal analysis, diel patterns at different times of the year, and response to episodic disturbance events.

Methods: We have maintained a bi-weekly sampling of water from the pier at Pensacola Beach, Florida for three years to address seasonal variability. Diel patterns were investigated quarterly with increased sampling frequencies over a 50-hour time period. During the second and third years of sampling, in response to Hurricane Nate and Hurricane Michael, we increased sampling to once daily before, during, and after each storm. Data from these storm events was analyzed in comparison to previously defined values using the time series to determine the disturbance effect and recovery period specific to the microbial ecology due to these storms. The analytical methods used in samples follow standard protocols for bacterial production, bacterial cell counts, microbial diversity (16S rDNA), chlorophyll a, primary production, nutrients, temperature, salinity, and total suspended solids.

Results: This study is ongoing but seasonal patterns are recurring and mostly driven by temperature. Diel patterns are apparent but seasonal patterns and variations are still being determined. Hurricane Nate created an immediate response in microbial communities with an equally rapid recovery period. A similar pattern was observed with Hurricane Michael. Data analyses are continuing and will be presented.0

PW215 Evolution of bacterial resistance to last resort antibiotics during multi-generational exposure to environmental conditions

Faisal Ahmad Khan[1], Aminur Rahman[1], Bo Söderquist[2], Jana Jass[1]

[1]School of Science and Technology, The Life Science Center - Biology, Örebro University, Örebro, Sweden, Örebro, Sweden
[2]School of Medical Sciences, Örebro University, Örebro, Sweden, Örebro, Sweden

Background: Resistance to last-resort antibiotics is increasing worldwide. The environment plays an important role in the spread of antibiotic resistance due to the presence of anthropogenic contaminants. Recent studies have shown that bacteria can develop de-novo antibiotic resistance during persistent exposure to stress. However, these studies were performed under optimal growth conditions, thus extrapolating the results to environmental conditions.

Objectives: The objective was to study the evolution of resistance to six β-lactam antibiotics in Klebsiella oxytoca after long-term exposure to environmental waters at two different temperatures.

Methods: The environmental water was collected from a river receiving effluent from a wastewater treatment plant in Sweden. Quadruplicate lineages of K. oxytoca were grown in filter-sterilized water supplemented with 1% tryptone for 600 generations at 25oC and 17oC, and with and without sub-minimum inhibitory concentration (MIC) of meropenem. The evolved lineages were screened for altered susceptibility to carbapenems (meropenem, imipenem) and cephalosporins (cefepime, ceftazidime, cefoxitin, cefotaxime) using disc-diffusion and gradient methods.

Results: In most lineages, resistance to carbapenems remained unchanged. However, a moderate increase in meropenem resistance was observed in lineages exposed to meropenem at 25oC. At 25oC, all lineages showed >4-fold increase in MIC to ceftazidime. Increased resistance to cephalosporins was commonly observed both at 25oC and 17oC, however, resistance evolved more rapidly at 25oC. The results showed that an increase of temperature is associated with increased evolution of bacterial resistance to antibiotics, thus play a pivotal role to accelerate emergence of antibiotic resistance in the environment.

PW216 Potential of propolis in the control of diseases caused by Oomycetes

Anđela Miljanović[1], Ana Bielen[1], Ana-Marija Iveljić[2], Dora Pavić[1], Ivana Maguire[3], Maja Dent[4]

[1]*Faculty of Food Technology and Biotechnology, Department for Biochemical Engineering, Zagreb, Croatia*
[2]*Faculty of Food Technology and Biotechnology, Zagreb, Croatia*
[3]*Faculty of Science, Department of Biology, Zagreb, Croatia*
[4]*Faculty of Food Technology and Biotechnology, Department of Chemistry and Biochemistry, Zagreb, Croatia*

Background: Oomycetes are globally important pathogens of plants and animals, causing significant economic losses in agriculture and aquaculture. Since current methods for their treatment include chemicals harmful to humans and the environment, new environmentally friendly methods for their control should be developed.

Objectives: The aim of this study was to examine anti-oomycete effects of propolis, a natural substance with known antimicrobial activity but poorly investigated anti-oomycete potential.

Methods: Two propolis formulations were used: P1 and P2, and their chemical composition was determined spectrophotometrically. Their anti-oomycete activity was determined using an in vitro plate assay and a range of pathogenic oomycetes that parasitize on animals (*Aphanomyces astaci*, crayfish pathogen, and *Saprolegnia parasitica*, parasite of salmonid fish) and plants (*Phytophora cactorum* and *P. plurivora*).

Results: Both propolis samples contained high, mg / mL concentrations of bioactive compounds: flavonoids, total phenols, hydroxyacetic acids and flavanols. Accordingly, high inhibitory activity of propolis was demonstrated against all tested Oomycetes in vitro: mycelium growth was slowed down from 30 % to 75 % in the presence of propolis formulations and the degree of inhibition was positively correlated with the concentration of flavonoids in the samples. In conclusion, this is one of the first studies that demonstrates strong inhibitory effect of propolis towards pathogenic Oomycetes. In the future, the effect of propolis should be tested in vivo, e.g. as food additive in freshwater aquaculture facilities.

PW217 Evaluation of quantitative PCR technologies for the detection and quantification of AmpC beta-lactamase antibiotic resistance genes from environmental samples

Roelof Coertze[1], Carlos Bezuidenhout[1]

[1]*North-West University, Potchefstroom Campus, Microbiology, Potchefstroom, South Africa*

Background: Due to increasing morbidity and mortality rates in humans due to bacterial infections associated with plasmid-mediated AmpC genes (pAmpCs), it is imperative that rapid, affordable and reliable detection methods for pAmpCs be developed for aquatic environments where the extent of AmpC prevalence is unknown and the implications uncertain.

Objectives: The aim of this study was to evaluate the use of quantitative PCR and digital PCR for the quantification of pAmpCs from mixed enriched bacterial DNA with the goal of applying these methods for the analysis of isolated eDNA.

Methods: Major AmpC gene groups that were analysed were ACC, ACT/MIR, BIL/LAT/CMY, DHA, FOX and MOX/CMY. All these genes were predesigned for clinical analysis by a commercial assay. A real-time PCR presence/absence protocol was used initially for detection. This was followed by quantification using qPCR and 3D chip-based digital PCR using QuantStudio hardware.

Results: This study demonstrated that the results of qPCR and dPCR for the quantification of pAmpCs from mixed bacterial samples were comparable ($p > 0.05$). Although no clear advantage of either of the two methods was observed, the use of any method depends on the nature of the study and time and financial resources available. However, the use of dPCR is not yet practical for the analysis of environmental samples, but further development in dPCR technologies by increasing the dynamic range and increasing the sample output could have positive implications for the detection and quantification of pAmpCs from environmental samples in the future.

PW218 Environmental antimony transformation by indigenous Sb(V) respiring microbiota

Chuanyong Jing[1], Li Ye[1], Li Yan[1]

[1]*Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing, China*

Background: The anthropogenic antimony (Sb) pollution is a worldwide problem. Nevertheless, compared with arsenic, an element in the same group in the periodic table, Sb biotransformation and mobility in the environment are much less understood.

In the anaerobic subsurface, microbial respiratory reduction is often accelerated by electron shuttles, such as quinone moieties in humic substances. However, little is known about the role of electron shuttling in Sb(V) respiring reduction and the consequent Sb mobilization.

Objectives: The objective of this study is to understand Sb biotransformation and mobilization in the subsurface by indigenous Sb(V) reducing microbiota (SbRM).

Methods: The soil samples were collected near an active Sb mine, and anaerobic enrichment was performed in a glove box. The DNA extraction, 16S rRNA gene sequencing and analysis were performed following the standard protocols. Chemical speciation analysis was conducted using multiple complementation characterization techniques for aqueous and solid samples.

Results: The components and structure of the enriched microbiota in the five generations were analyzed accordingly using high-throughput sequencing technology. Inspired by the extensive studies on the As(V) reducing mechanism under anaerobic conditions, we hypothesized that Sb(V) reduction by SbRM may follow a similar respiration pathway. Our results suggest that Sb(V) was used as an electron acceptor for the SbRM anaerobic respiratory growth. The Sb speciation in soils was characterized using XANES. The LCF results show that the soil contained 11% Sb2S3. Sulfate and ADQS amendments did not further increase the Sb(III) content, but enhanced the Sb2S3 concentration to 29-31%.

PW219 Quantifying the Transport of Tracer Phages in Soil

Xin You[1], Antonis Chatzinotas[1], Florian Schattenberg[1], Susann Müller[1], Hauke Harms[1], Lukas Wick[1]

[1]*Helmholtz Centre for Environmental Research - UFZ, Department of Environmental Microbiology, Leipzig, Germany*

Background: Bacteriophages (or short phages) are viruses that specifically infect their host bacteria. They are nanometer scale colloidal particles and can penetrate soils through natural water discharge. Marine phages are considered as ideal tracers for colloidal transport and water flow in terrestrial ecosystem, because of the absence of their marine host. However, the highly heterogeneous structure of soil may deactivate and sorb transported phages and thus limit the use of phage tracers in soil systems.

Objectives: In this study we strive to quantify the transport of different tracer phages in saturated soil and to compare phage breakthrough to the transport of non-reactive solute tracers.

Methods: Three lytic tracer phages of different morphology and phys.-chem. surface properties were applied in saturated laboratory soil columns; i.e. hydrophobic *E. coli* phage T4 and two hydrophilic marine *Pseudoalteromonas* phage HS2 and HM1. Bromide was used as non-reactive tracer. Breakthrough of phages and bromide are quantified by the plaque forming unit (PFU) and ion chromatography, resp.

Results: We found significant breakthrough of all phages in soil filled saturated columns. Data show that T4 phage has the highest PFU-based mass recovery (84%), followed by HS2 (71%) and HM1 (46%), while the non-reactive bromide tracers all reached approximately 100% mass recovery. Our data suggest that phages are good tracers in complex soil systems.

PW220 Antimicrobial resistance genes and their host bacteria in human impacted environments in West Africa

Anniina Sarekoski[1], Isidore Bonkoungou[2], Kaisa Haukka[1], Marko Virta[1]

[1]*University of Helsinki, Department of Microbiology, Helsinki, Finland*
[2]*University Ouaga I, Pr Joseph Ki-Zerbo, Bacteriology-Virology, Burkina Faso*

Background: Antimicrobial resistance (AMR) is a rapidly increasing global threat to human health and every year 700 000 people die from infections caused by bacteria resistant to antimicrobials. AMR poses a threat not only in health care settings but also in the community and livestock, and thus a One Health approach is needed. AMR has especially increased in developing countries due to the lack of control in antibiotic use and poor infrastructures. However, in some developing areas, such as in West Africa, AMR has not yet been extensively studied.

Objectives: I focus on identifying AMR genes in human impacted rural and urban water environments, such as hospital, industrial and open market wastewaters, animal slaughter sites, canals and ditch water. More precisely, I determine the host range of AMR genes found in these environments and concentrate on AMR-gene-carrying pathogens. I want to understand how human impact contributes to these findings by comparing rural and urban areas and different sampling sites. Thus, the study aims to trace the flow and evolution of AMR genes in West Africa.

Methods: AMR genes are detected with SmartChip qPCR array analysis. Further, the host range of few carefully selected AMR genes is profiled by using epicPCR (emulsion paired isolation and concatenation PCR). EpicPCR is a novel high-throughput culture-free method that links functional genes and phylogenetic markers in single cell resolution.

Results: I am expecting a high abundance of AMR genes especially in wastewater and differences in the spectrum of AMR-gene-carrying bacteria between rural and urban areas.

PW221 The Use of epicPCR to Determine the Host Range of Antimicrobial Resistance Genes in Manure and Manure-Fertilized Soils in Finnish Dairy Farms

Minna Maunula[1], Johanna Muurinen[2], Katariina Pärnänen[1], Jenni Hultman[1], Marko Virta[1]

[1]*University of Helsinki, Department of Microbiology, Helsinki, Finland*
[2]*Purdue University, Department of Animal Sciences, United States*

Background: Modern animal production relies on the use of antimicrobials, which increases the emergence of antimicrobial resistance. Storage and use of animal manure as a fertilizer is a potential route for the dissemination of antimicrobial resistance genes via horizontal gene transfer.

Objectives: The use of antimicrobials in animal production is more restricted in the EU than elsewhere in the world. Especially in Finnish animal husbandry the antimicrobials are used predominantly to treat infections. It has been shown that antimicrobial resistance genes are abundant in manure and the abundance increases during the winter storage. Therefore, the storage may enhance the effect of manure application, which is a potential route to horizontally transfer the antimicrobial resistance genes in manure to soil bacteria. Harvested feed, in turn, is a potential route from soil bacteria to gut microbes in livestock. The question remains, what is the host range of these genes? Are the species pathogenic and are the genes transferred horizontally?

Methods: We use culture-independent epicPCR to investigate the host range of aminoglycoside, beta-lactam and tetracycline resistance genes in fresh and stored manure and soil after fertilization in two Finnish dairy farms. The method links the antimicrobial resistance gene to the phylogenetic marker gene together, which gives answers to aforementioned questions.

Results: Early results show, that all the three tested genes are found in some manure and soil samples. There is a difference between the farms whether the gene is detected or not. After sequencing we will discover the host range of these genes.

PW222 Metabolic and Genetic Characterization of Fluorescent Pseudomonads exhibiting bio-control potential and bio-fertilizer traits

Izzah Shahid[1;2;3], Samina Mehnaz[2], Jun Han[3], Christoph H. Borchers[3], Darryl B. Hardie[3], Deeba Noreen Baig[2], Kauser Abdulla Malik[2]

[1]*University of Central Punjab, Lahore, Pakistan*
[2]*Forman Christian College (A Chartered University), Lahore, Pakistan*
[3]*UVic Genome BC Proteomics Centre, Victoria, Canada*

Background: Fluorescent pseudomonads are considered as key players in sustainable agriculture for their biofertilizer and biocontrol potential. However, very few studies comprehensively characterize the putative biocontrol genes, antagonistic metabolites, and biofertilizer traits, simultaneously.

Objectives: This study evaluated four different species of *Pseudomonas* for their *in vitro* biocontrol potential against fungal phytopathogens, role in wheat growth promotion, and characterization of antimicrobial compounds.

Methods: Molecular characterization of *Pseudomonas* sp. isolates was based on 16S rRNA gene while antifungal activity of these strains was determined by agar well diffusion method. Secondary metabolites were subjected to LC/ESI-MS/MS for confirmation of compounds and $[M + H]^+$ ions were monitored. All strains were also screened for plant growth promoting traits and plant experiments were conducted on wheat in climate control room.

Results: On average, all strains exhibited 0.8 cm inhibition zones against the causal agents of root rot, seedling blight, red and stem rot. All eleven strains showed the production of six phenazine derivatives, 2-acetamidophenol, pyochelin, and *N*-acylhomoserine lactones while pyrrolnitrin, WLIP, 2-hydroxyphenazine and 2,8-dihydroxyphenazine were unique to *P. aurantiaca* strains only. Production of three new ortho-dialkyl-substituted aromatic acids; Lahorenoic acid A, B, C was also demonstrated by *Pseudomonas chlororaphis.* IAA, HCN, extracellular enzymes production, phosphate and zinc solubilization were observed by many strains and *P. aurantiaca* strains; ARS-38, RP-4, PB-St2 and *P. fluorescens* RS-1 considerably increased wheat shoots and roots biomass and lengths. These findings indicate the potential of these *Pseudomonas* strains to be used as user-friendly single-strain bioinoculums with multifaceted biocontrol and biofertilizer traits.

PW223 epicPCR - a novel culture-free method to study antimicrobial resistance in the environment

Jesse Vänttinen[1], Jenni Hultman[1], Manu Tamminen[2], Marko Virta[1]

[1]*University of Helsinki, Department of Microbiology, Helsinki, Finland*
[2]*University of Turku, Department of Biology*

Background: EpicPCR, short for Emulsion Paired Isolation and Concatenation PCR, is a novel emulsion-based method, which can be used to answer the question of 'who does what' in a microbial community. In epicPCR a culture-free, emulsion-based fusion PCR -technique is used to link phylogenetical markers, such as 16S ribosomal RNA gene, to functional target genes, such as antimicrobial resistance genes. This provides a throughput of hundreds of thousands of cells with relatively low costs. EpicPCR is a technique that has been used successfully in published research. However, the exact number of cells to give a positive signal in epicPCR is still in question and the PCR conditions can be optimized.

Objectives: My aim is to optimize the method's PCR-reagent concentrations, as well as the PCR conditions, including annealing temperatures and the amount of cycles used.

Methods: The methods used in the optimization process include temperature gradient PCR, epicPCR, agarose gel electrophoresis and Illumina MiSeq sequencing.

Results: epicPCR is a versatile method that can be used to study, for instance, carriers of antimicrobial resistance genes. With the utilized optimization approaches qualitative aspects of epicPCR can be determined. In addition, first results indicate increased sensitivity with these approaches.

PW224 Exploring the Quorum Quenching activity of Antarctic Bacteria

Cristina García-Aljaro[1], Judit Martínez-González[2], Roger Tiell[1], Carlos Angulo-Preckler[3], Conxita Avila[3], Elisenda Ballesté[1]

[1]*University of Barcelona, Department of Genetics, Microbiology and Statistics, Barcelona, Spain*
[2]*University of Barcelona, Genetics, Microbiology and Statistics, Barcelona, Spain*
[3]*University of Barcelona, Department of Evolutionary Biology Ecology and Environmental Sciences, and Biodiversity Research Institute (IrBIO), Barcelona, Spain*

Background: Polar marine ecosystems remain underexplored shielding unknown species. This species may produce natural compounds to allow their survival becoming a source of molecules which can be applied in medicine or biotechnology.

Objectives: The objective of this work was to isolate, identify and characterize bacteria associated to polar marine water and different Antarctic invertebrates, with special focus on the presence of quorum quenching (QQ) activity.

Methods: Bacteria were isolated from porifera (*Axinella crinita*, *Axinella polypoides*, *Dendrilla antarctica*, *Hemigellius pilosus*, *Kirkpatrickia variolosa*, *Mycale acerata, Sphaerotylus antarcticus*), cnidaria (*Alcyonium haddoni*), tunicate (*Aplidium sp.*), equinodermata (*Sterechinus neumayery*) and marine water, in Deception Island, South Shetlands Island (Anctarctica), in marine R2A and WPCA. The isolates were identified on the basis of their 16S rRNA gene sequence. The presence of QQ activity was determined using the well diffusion assay using bacteria cultures supernatants previously inoculated with different acyl-homoserine lactones (C6-HSL and C12-HSL) and the *Chromobacterium violaceum* CV26 and *C. violaceum* VIR07 strains as sensor strains.

Results: The phylum Proteobacteria was the most abundant, although isolates belonging to Firmicutes, Actinobacteria and Bacteroidetes were also detected. QQ activity was detected in the following phyla and genera: Proteobacteria (*Psychrobacter, Pseudoalteromonas, Pseudomonas,* Sphingomonas), Actinobacteria (*Arthrobacter, Rhodococcus*), Bacteroidetes (*Flavobacterium, and Polaribacter*), and Firmicutes (*Staphylococcus, Bacillus*). The majority of the isolates presented activity against the C12-HSL, whereas only *Polaribacter* presented activity against both the C6-HSL and the C12-HSL. However, the molecule implied in the QQ has still to be identified, indicating that Antarctic ecosystems are potential sources of novel QQ enzymes.

PW225 Fecal pollution can explain antibiotic resistance gene abundances in anthropogenically impacted environments

Antti Karkman[1], Katariina Pärnänen[1], Joakim Larsson[2;3]

[1]*Univeristy of Helsinki, Department of Microbiology, Helsinki, Finland*
[2]*University of Gothenburg, Department of Infectious Diseases, Gothenburg, Sweden*
[3]*Univeristy of Gothenburg, Center for Antibiotic Resistance research (CARe) , Gothenburg, Sweden*

Background: Discharge of treated sewage leads to release of antibiotic resistant bacteria, resistance genes and antibiotic residues to the environment. However, it is unclear whether increased abundance of antibiotic resistance genes in sewage and sewage-impacted environments is due to on-site selection pressure by residual antibiotics, or is simply a result of fecal contamination with resistant bacteria.

Objectives: Our goal was to develop an approach that can help in disentangling environmental on-site selection and/or horizontal dissemination of antibiotic resistance genes from passive dissemination/enrichment by fecal pollution.

Methods: Using crAssphage as a marker of human fecal contamination, we analyzed the extent of fecal pollution in human impacted environments using publicly available metagenomic data and correlated it with relative resistance gene abundance.

Results: We find that the presence of resistance genes can largely be explained by fecal pollution, with no clear signs of selection in the studied environments, with the exception of environments polluted by very high levels of antibiotics from drug manufacturing, where selection is evident. Our results demonstrate the necessity to consider fecal pollution levels to avoid making erroneous assumptions regarding environmental selection of antibiotic resistance. The method used provides a framework to help disentangling dissemination of resistant human fecal bacteria from the possible selection and horizontal gene transfer of resistance genes in the environment. Our results bring insight to the dynamics of ARGs in sewage receiving environments and highlight the importance of measuring fecal pollution when assessing the selection and dissemination patterns of antibiotic resistance genes in the environment.

PW226 Impact of prebiotics and low-protein nutricional management in gut microbiota and progression of chronic kidney disease

Waldo Díaz-Vásquez[1], Valentina Fernández Yáñez[1], Francisca Peña[1], Melissa Díaz Roblero[1], Javiera Portus[1]

[1]San Sebastián University, Facultad de Ciencias para el Cuidado de la Salud, Molecular Microbiology and Food Resarch Laboratory, Providencia, Chile

Background: Chronic kidney disease (CKD) has five stages, patients in end stage (ES-CKD) must be subjected to -hemodialysis, peritoneal dialysis or kidney transplantation. Chile has ~21,000 patients on dialysis (22% of annual public health budget). Intestinal microbiota (IM) can remain in stable -homeostasis- or altered -dysbiosis- conditions, latter is associated with CKD course. IM aminoacid and urea degradation promote uremic toxins generation P-cresyl sulphate, producing kidney fibrosis; and indoxyl sulfate generating oxidative stress in osteoblasts, decreasing the expression of PTH receptors, thus triggering loss of epithelium integrity, bacterial endotoxemia and gut inflammation.

Low-protein diet and prebiotics can reduce progression of CKD, uremic toxins generation, and have a positive effect on restitution of IM. Oligofructose, inulin and resistant starch significantly reduce p-cresyl production and gut inflammation. On the other hand, CKD of uncertain etiology occurs by heavy metal(oid)s and antibiotics exposure. In Chile lead, arsenic, cadmium and mercury are present in tap water.

Objectives: To characterize the composition and diversity of the IM upon the administration of a low-protein diet supplemented with prebiotics in patients with CKD under predialysis stages (G3A, G3B and G4).

Methods: Voluntarily were evaluated by 6 months. Renal function parameters (eGFR, MAU and CRPu) were determined. qPCR and 16S metagenomic were used for IM analysis on Stool samples. Viable microorganisms counting, bacteria isolation and metal(oid)s / antibiotics resistance was also evaluated.

Results: Low-protein diet supplemented by inulin and oligofructose prebiotic cause partial restoration of IM, metal(oid)s and antibiotics resistant bacteria were augmented in CKD stool samples.

PW227 Microbial interactions in marine sediments exhibiting long-distance electron transport by cable bacteria

Jeanine S. Geelhoed[1], Cheryl Karman[2], Stanislav Trashin[2], Silvia Hidalgo-Martinez[1], Sebastiaan van de Velde[3], Karolien de Wael[2], Filip J.R. Meysman[1;4]

[1]*University of Antwerp, Department of Biology, Antwerp, Belgium*
[2]*University of Antwerp, Department of Chemistry, Antwerp, Belgium*
[3]*University of California, Department of Earth Sciences, Riverside, United States*
[4]*Delft University of Technology, Department of Biotechnology, Delft, Netherlands*

Background: Cable bacteria are long filamentous bacteria that can transport electrons over centimetre-long distances in sediments. The metabolic activity of cable bacteria strongly affects the sediment geochemistry, changing the availability of lithotrophic substrates, and may perhaps also impact the microbial community through interspecies electron transfer.

Objectives: To study the impact of cable bacteria activity on the residing microbial community in marine sediments.

Methods: Marine sediment incubations with and without cable bacteria were characterised by porewater analysis, and 16S-amplicon and whole-metagenome sequencing. We also performed physiological studies and cyclic voltammetry with cultures enriched from sediment.

Results: Gammaproteobacteria were the most abundant phylum in surface sediment without cable bacteria, while Alphaproteobacteria contributed ~50% of the 16S-amplicon sequences in the presence of cable bacteria. This shift may be explained by the increased concentration of porewater Fe^{2+} caused by cable bacteria activity, if Fe^{2+} is used as electron donor by the Alphaproteobacteria. Alternatively, the shift could also indicate direct interspecies electron transfer between Alphaproteobacteria and cable bacteria. To investigate this, we examined the metagenomics-resolved genome of the most abundant Alphaproteobacterium, affiliated to the Rhodospirillales, for the encoded metabolic capacity and potential for extracellular electron transfer. In addition, representative cultures were tested for the utilization of Fe(II) and cyclic voltammetry was employed to study the ability for extracellular electron transfer. Together with a quantitative evaluation of the flux of Fe(II) and the current density generated by the cable bacteria, these data provide insight into why the Alphaproteobacteria thrive in the presence of cable bacteria.

PW228 Understanding bacterial colonisation and biofilm development dynamics in water biofilters

Marta Vignola[1], Cindy Smith[1], William Sloan[1]

[1]*University of Glasgow, Infrastructure and Environment*

Background: Filtration through porous media (sand or Granular Activated Carbon-GAC) is a conventional treatment widely used to physically remove suspended solids and soluble organic matter from drinking water. Highly diverse microbial communities have been found populating these systems; however, the mechanisms driving the development and the behavior of such communities are still poorly understood.

Objectives: In this study, we studied the formation of the biofilm in lab-scale biofilters, packed with GAC, with three different bed's depths: 30cm, 60cm, 90cm.

Methods: Filters were set up in triplicates for each depth, fed with reservoir water in down-flow mode (1mL/min) and operated for 82 days. Samples of the influent and effluent water were collected weekly and analysed for ATP content, total and intact cells count via flow-cytometry. Additional reactors were operated and sacrificed throughout the experiment for biofilm quantification on grains, via ATP.

Results: Total and Intact suspended cells were removed to the same extent from the influent water in all the three sets of filters, 42%±16% on average. No statistically significant difference was observed between the three effluents, suggesting that particles and cells removal occur mainly in the first 20cm of the filter's bed. Indeed, ATP analyses showed that 62% of the total cells were attached to the first 15cm of the filter.

Calculation based on the mass balance of cells entering and leaving the filters throughout the experiment showed high cells decay rates, suggesting that complex ecological mechanisms might be involved in the control of filter's biofilm growth and development.

PW229 Impact and risk of hazardous dietary substances on human microbiota and its role in several dysbiosis phenotypes

Soumi de Montijo[1], Ana Rivas[2], Inmaculada Acuña[3], Antonio Suárez[3], Margarita Aguilera[4]

[1]*University of Granada, Department of Microbiology, Faculty of Pharmacy, Spain*
[2]*University of Granada, Department of Nutrition, Faculty of Pharmacy, Spain*
[3]*University of Granada and CIBM, Department of Biochemistry, Faculty of Pharmacy, Spain*
[4]*University of Granada and CIBM, Department of Microbiology, Faculty of Pharmacy, Spain*

Background: Factors and subtances that affect human microbiota eubiosis should be studied with a multidisciplinary approach as it is a complex field of health research. Interaction among distinct scientific disciplines as microbiology, nutrition, toxicology and personalised medicine are needed. Moreover, multiple technologies, communities and professional domains should converge in order to obtain relevant outcomes.

Objectives: To obtain upmost information about human microbiota variability associated and/or caused by diet hazardous substances exposure and consumption.

To analyse the ability of modulation or intervention with nutraceuticals in order to recover the physiological balanced status.

Methods: A systematic review method to be applied in different searching engine databases (Pubmed, Scifinder, Web of Science, Scopus, Embase) has been designed with specific key words (microbiota, endocrine disruptors, obesity, insuline resistence, dyslipidemia, hypertension, sythoms of metabolic syndrome, infertility, probiotics, interventions, omics technologies). Specific exclusion and inclusion criteria, and categorisation of studies.have been applied in order to obtain relevant documents for the holistic analysis of human microbiota.

Results: A decision tree scheme and PRISMA information flow diagram for the systematic review that integrate whole manuscripts with relevant outcomes will be presented. The list of Authors, date, country, sampling and method applied, study aim, findings, and main conclusion will be clustered and quantified in order to evaluated and discussed to show the general conclusions. An harmonised characterisation of relevant results will be presented in order to contribute to elucidate the role of human microbiota in new common multifactorial misbalance health area that remain still poorly understood.

PW230 Dual species calcium carbonate-biofilm formation by alkaline generating Lysinibacillus boronitolerans YS11 and alkaliphilic Bacillus sp. AK13

YunSuk Lee[1], Woojun Park[1]

[1]Korea University, Department of Environmental Science and Ecological Engineering, Seoul, Korea, Rep. of South

Background: Microbially induced $CaCO_3$ precipitation (MICP) is a process where microbes induce condition favorable for $CaCO_3$ formation through metabolic activities by increasing the pH or carbonate ions when calcium is near.

Objectives: Molecular and ecological basis of $CaCO_3$ precipitating (CCP) bacteria has been poorly illuminated. This work describes molecular details of alkaline-generating CCP bacteria and ecological aspects of MICP for the first time.

Methods: The genome sequencing and RNA sequencing of *Lysinibacillus boronitolerans* YS11 was conducted using Pac Bio RSII Single Molecule Real Time (SMRT) sequencing method and Illumina HiSeq 2500 platform, resepectively. Newly isolated alkaliphilic *Bacillus* sp. AK13 was identified based on 16S rRNA sequencing. Morphological analysis of $CaCO_3$ was examined by field emssion-scanning electron microscopy and Fourier-transform infrared spectroscopy. Biofilms were quantified by crystal violet staining assay, and confocal laser scanning microscopy was used for visualization of biofilm with FilmTracer™ SYPRO® Ruby biofilm matrix dye. Branched chain fatty acid in membrane was evaluated by fatty acid methyl ester analysis.

Results: We showed that alkaline generation by deamination of amino acids is a driving force toward MICP using alkalitolerant *L. boronitolerans* YS11 as a model species of non-ureolytic CCP bacteria. This alkaline generation also facilitates the growth of neighboring alkaliphilic *Bacillus* sp. AK13, which could alter characteristics of MICP by changing the size and shape of $CaCO_3$ minerals. Furthermore, we showed that early precipitated $CaCO_3$ modifies membrane rigidity of YS11 strain via upregulation of branched chain fatty acid synthesis.

PW231 gc-ms and 1h-nmr based metabolomic insight of a chronically petroleum hydrocarbon farmland at ngia ama, tombia, niger delta

Emmanuel Oliver Fenibo[1], Chioma Blaise Chikere[2], khayalethu nthushelo[3], onyewhuchi akaranta[4]

[1]*world bank africa centre of excellence, centre for oilfield chemical research, environmental microbiology, port harcourt, Nigeria*
[2]*University of Port Harcourt, microbiology, Port Harcourt, Nigeria*
[3]*university of south africa, agriculture, Johannesburg, South Africa*
[4]*world bank africa centre of excellence, centre for oilfield chemical research, chemistry, Port Harcourt, Nigeria*

Background: Ngia Ama (4.7947°N, 6.6831°E) has been hitherto used for artisanal refining activities. The site used for this study is chronically polluted to such an extent that there are no vegetation at the affected site. The restoration of the polluted site requires bioremediation which currently is been intensely driven by all levels of omics disciplines including metabolomics. The latter is a post-genomic tool with immense and versatile value that reveals unknown biochemical modes of actions of environmental stressors and how organisms respond to these pollutants.

Objectives: The aim of this study is to elucidate metabolomics fingerprinting as a biomarker of *in-situ* microbial bioremediation process.

Methods: The total petroleum hydrocarbon was determined by gas chromatography-mass spectrometry (GC-MS) to ascertain the level of hydrocarbon pollution. Secondary metabolites were identified by GC-MS and proton nuclear magnetic resonance (^{1}H-NMR). Web-based tools (the chemical translation service, MetLin), softwares (MetaboAnalyst 4, NMRshiftDB2) and databases (Kyoto encyclopedia of genes and genome (KEGG), MetaCyc) were used to analyse the GC-MS and ^{1}H-NMR data with a view to identifying the environmental metabolites, pathways and hydrocarbon degrading microorganisms.

Results: The TPH was 490,630 ppm (0-30 cm depth). Thirty five pathways involving aromatic hydrocarbons (PAHs and BTEX), carbazole, chloro-hydrocarbons with associated microorganisms (*Pseudomonas*, *Burkholderia*, *Sphingomonas*, *Bacillus* etc) were identified. The elucidated metabolic pathways show that despite the high level of petroleum hydrocarbon contamination (with heavy fractions) in the farmland, intrinsic microbial bioremediation is evident.

PW232 Ciliates facilitate the transfer of a vancomycin resistance gene in Enterococcus faecalis

Temilola Olanrewaju[1], James Dooley[2], Joerg Arnscheidt[3]

[1]*Ulster University, Geography and Environmental Sciences, Coleraine, United Kingdom*
[2]*Ulster University, Biomedical Sciences, Coleraine, United Kingdom*
[3]*Ulster University, Geography and Environmental Sciences, Coleraine, United Kingdom*

Background: Enterococci are ubiquitous in aquatic environments and are highly efficient at acquiring new antimicrobial resistance (AMR) genes. Grazing by ciliates on enterococci could promote bacterial aggregation as a precondition for conjugal AMR gene transfer.

Objectives: This study investigated the propensity of freshwater ciliates to conjugally promote vancomycin resistance gene transfer between *Enterococcus faecalis* strains within ciliate vesicles.

Methods: Conjugation treatments containing a mix of an equal 10^9 cfu/ml concentration of a vancomycin-resistant *E. faecalis* donor strain and a rifampicin-resistant *E. faecalis* recipient strain were prepared in Page's amoeba saline media. Treatments were incubated with live or heat-killed ciliate, *Tetrahymena pyriformis,* for 24 h at 20 ^{o}C. Control treatments contained no ciliates. The transfer of a *vanA* antimicrobial resistance gene from donor to recipient *E. faecalis* strain was detected by isolating transconjugants on antibiotic selection agar plates. The identify and similarity of the *vanA* gene present in the donor strain and transconjugants were confirmed through gene amplification and sequencing.

Results: Vancomycin-resistant transconjugants were detected in conjugation treatments. The observed difference in the frequency of gene transfer between treatments with live ciliates and controls was significant. However, there was no significant difference in transconjugant numbers between treatments with dead ciliates and control treatments, suggesting that gene transfer in *T. pyriformis* was facilitated by intracellular processes.

Conclusions Actively grazing ciliates facilitated the conjugal transfer of a *vanA* gene in *Enterococcus faecalis.* This appears to be the first evidence of promotion of AMR gene transfer in Gram positive bacteria through ciliates.

PW233 Bioinformatic workflow in environmental metaproteomics: metagenome-derived protein databases and their biological interpretation discrepancies

Augustin Geron[1;2], Johannes Werner[3], ruddy wattiez[2], Philippe Lebaron[4], Sabine Matallana-Surget[1]

[1]*University of Stirling, Division of Biological and Environmental Sciences, Stirling, United Kingdom*
[2]*University of Mons, Proteomic and Microbiology Department, Mons, Belgium*
[3]*Leibniz Institute of Baltic Sea Research, Department of Biological Oceanography, Rostock-Warnemünde, Germany*
[4]*Observatoire Océanologique de Banyuls-sur-Mer, Banyuls-sur-Mer, France*

Background: Metaproteomics is a powerful tool to study the diversity of environmental samples, linking microbial community structure and microbial processes, providing key insights into the functioning of a given ecosystem. However, the power of metaproteomics is still hampered by the inability of conventional proteomics bioinformatic workflow to deal with the inherent complexity of environmental samples. How to improve and standardize query protein database creation and protein sequences annotation are questions of immediate urgency.

Objectives: In our study we compared the metaproteomics networks obtained by using three query protein databases derived from the same metagenome. The three databases compared in this study were the following (i) non-assembled metagenome, (ii) assembled metagenome and (iii) taxonomy derived databases.

Methods: For this database comparison, we used both gel-free and gel-based proteomics approaches, on marine sea surface samples. The three search protein databases were obtained using mPies, an in-house program, allowing the automatization of database creation and a consensus annotation based on the Last Common Ancestor (LCA) principle for both the taxonomy and protein function.

Results: We demonstrated that the choice of the database strongly affects the total number of identified proteins and significantly influences the biological interpretation for both the structure and functioning of the microbial community. This work emphasized the importance of the design and annotation of the search protein databases, providing the advantages and limits of each approach and their impact on the understanding of a given ecosystem.

PW234 Antibiotic resistance genes in bacterial communities in akaki river, Ethiopia

Berhanu Sahilu[1], yimtubezinash woldeammanuel mulate[1], daniel asrat[1], Adane Mihret[2], Abraham Aseffa[2], per-erik olsson[3], Jana Jass[3]

[1]Addis Ababa University college of Health sciences, አዲስ አበባ, Ethiopia
[2]Armauer Hansen Research Institute, Ethiopia
[3]School of Science and Technology, Örebro Life Science Center-Biology, Örebro University, Sweden

Background: The emergence of antibiotic resistant pathogens is a global public health concern. Continual fecal discharge into aquatic environments with high levels of chemical and pharmaceutical pollutants is creating fertile conditions and selective pressure for generating antibiotic resistant pathogens.

Objective: The aim of the study was to investigate the distribution of antibiotic resistant genes (ARGs) and pathogenic bacteria in the Akaki river, Ethiopia.

Methods: Water samples were collected from five sites along the Akaki river for total DNA extraction. The presence of 87 ARGs and pathogenic bacteria were evaluated using qPCR analysis.

Results: The water samples contained genes associated with fluoroquinolone, aminoglycoside, β-lactamase, macrolide, multidrug resistance and tetracycline efflux pumps and vancomycin resistance. They were persistent at various sampling points in the river with some spatial variation. The majority of ARGs were identified from sites in close proximity to anthropogenic activities including hospitals and industries. The middle catchment of the river was positive for 71-75% of targeted ARGs. The genes *AAC(6)-Ib-cr*, *aadA1*, *OXA-10*, *QnrS*, *mefA* and *tetA* were detected in all sampling sites. The beta-lactam resistance genes, *NDM* and *OXA-55,* were identified from only one site near the industrial zone. In addition, *Escherichia coli, Salmonella enterica*, *Vibrio cholerae* and other potentially pathogenic bacteria were detected in the water samples. As with the resistance genes, the middle catchment was the site where the majority of bacteria were identified.

Conclusion: Our findings indicate that the Akaki river is a potential hotspot for evolution and spread of ARGs and thus antibiotic resistant bacteria.

PW235 Temperature- and time-dependent microbial community dynamics modulates methane cycle in permafrost-affected soils

Nuri Myeong[1;2], Mincheol Kim[1]

[1]*Korea Polar Research Institute, Incheon, Korea, Rep. of South*
[2]*Chung-Ang University, Systems Microbial Ecology, ANSEONG,*

Background: Arctic permafrost soils store large amounts of SOC (soil organic carbon). Thawing permafrost promotes microbial degradation of SOC leading to the biogenic production of greenhouse gases such as carbon dioxide and methane. However, the dynamics of microbial communities over the course of methane production and oxidation during permafrost thawing remains poorly understood.

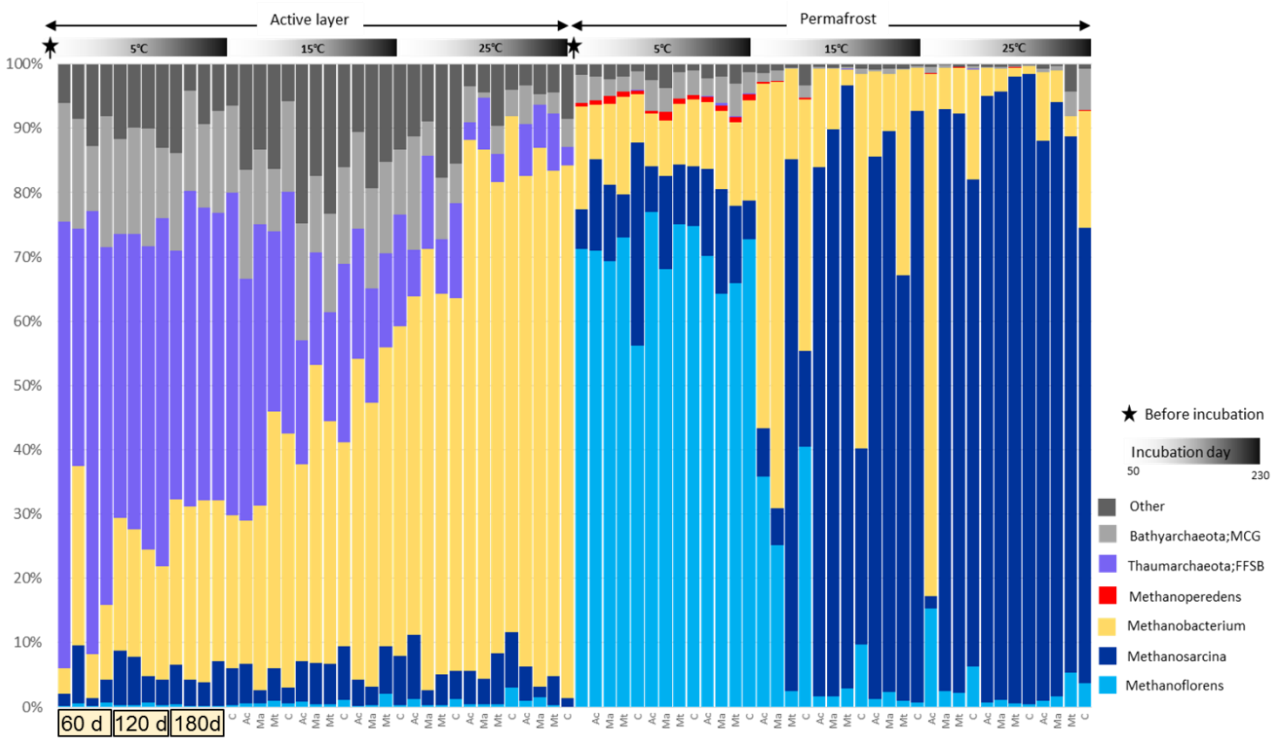
Objectives: This study aims to understand how methane cycle is modulated by microbial community changes driven by different temperatures and incubation times under anaerobic condition in permafrost-affect soils.

Methods: We anaerobically incubated Alaska Council soil cores. Both active layer and permafrost soils were incubated at 5℃, 15℃ and 25℃ with 13C-labelled aubstrate for 11 months. Soil DNA was extracted and then 16S rRNA gene targeting bacteria and archaea was sequenced using MiSeq platform.

Results: CH_4 concentration increased with time at all temperatures at both active layer and permafrost and, active layer show CH_4 produce more than permafrost at earlier stage of incubation. However, permafrost CH_4 produce increase abruptly later stage of incubation. Finally permafrost CH_4 concentration reached two times than active layer at all temperatures.

There are distinct patterns in community composition between active layer and permafrost. In permafrost, the relative abundance of '*Ca* Methanoflorens' maintained high at 5℃ but almost absent and replaced by Methanosarcina at 15℃ and 25℃. There is a clear shift in major methanogen lineage from low to higher temperatures.

These results help to understand the effect of different temperatures and incubation times on metabolic interactions between anaerobic microbes in Arctic tundra.


Active layer
Permafrost
5℃
15℃
25℃
5℃
15℃
25℃
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
60 d
120 d
180d
Before incubation
Incubation day
50
230
Other
Bathyarchaeota;MCG
Thaumarchaeota;FFSB
Methanoperedens
Methanobacterium
Methanosarcina
Methanoflorens

PW236 Biofilm formation vs stress factors: who can play at the game

Olga Iungin[1], Oleksandr Tashyrev[1]

[1]*D.K. Zabolotny Institute of Microbiology and Virology, Biology of Extremophilic Microorganisms, Kyiv,*

Background: The use of different chemicals for agriculture, industry and mining has caused pollution of agrarian soils which provokes changes in rhizosphere microflora. We have studied 10 bacterial strains isolated from a winter wheat Cd-polluted field in Ukraine by their taxonomic position, biochemical properties and resistance to 3 classes of toxicants: heavy metals (Cu^{2+}, Cd^{2+}), non-metals (perchlorate-ion), organic xenobiotic (1-chloro-4-nitrobenzene, CNB).

Objectives: To study the effect of toxicants on the biofilm–formation ability of individual strains and a mixed community.

Methods: Biofilm characteristics (total microcosm growth, biofilm strength and attachment to the microcosm walls) were studied by combined biofilm assay (n = 4) with four treatments including 100 mg/L Cu^{2+}, 25 mg/L Cd^{2+}, 300 mg/L ClO_4^-, and 100 mg/L CNB, with correlations and Principal component analysis (PCA) used to investigate data.

Results: We found that microbial community had a greater resistance to the toxicants compare to individual strains. The presence of heavy metals increased the strength of biofilms, and in most cases growth and biofilm strength were positively correlated. However, in the presence of Cd^{2+} this correlation was lost. Perchlorate affected bacteria, increasing mucus production and biofilm strength though it also reduced attachment levels. Finally, we also found that CNB could be used as a source of Carbon and energy during biofilm–formation.

PW237 Tracing long-distance aerial trajectories of aerial dissemination: The hunt for sources of Pseudomonas syringae deposited with snow at the Jungfraujoch high altitude meterological station

Cindy Morris[1], Odile Berge[2], Charlotte Chandeysson[2], Caroline Guilbaud[2], Cécile Monteil[2], Oddur Vilhelmsson[3], Margrét Auður Sigurbjörnsdóttir[3], Paula Darwin[4]

[1]*INRA-PACA, Plant Pathology Research Unit, Montfavet, France*
[2]*INRA, Plant Pathology Research Unit, Montfavet, France*
[3]*University of Akureyri, Natural Resource Sciences, Akureyri, Iceland*
[4]*University of Reading, Biological Sciences, Reading, United Kingdom*

Background: *Pseudomonas syringae* is a plant-associated, water-borne, aerially-disseminated bacterium with a role in the water cycle via its capacity to incite freezing of cloud water droplets and formation of precipitation. This fosters its fall-out from long distance travel in air masses and its ubiquity. Atmospheric interconnectivity between sources and sinks assures spread of plant-pathogenic variants to new regions or spread of atmospheric ice nucleating particles for precipitation at downwind sites. The ubiquity of *P. syringae* makes it technically difficult to precisely link sources and sinks and to trace its dissemination trajectories.

Objectives: Here we aimed to develop an approach to identify possible sources of *P. syringae* for sinks associated with known air mass trajectories and precipitation events and to provide evidence that corroborates the hypothesis that these sources and sinks are linked.

Methods: Strains of *P. syringae* from snowfall on 22/10/2014 on Jungfraujoch, CH (46.5475° N, 7.9792° E, 3500 m alt.) were isolated and genotyped. The backwards trajectory of the air mass that brought this snowfall was examined, and sites where this air mass neared the ground were identified. For one of these sites, in northern Iceland, strains of *P. syringae* from ground cover vegetation were isolated and characterized to search for clones of the 24 strains isolated from the Jungfraujoch snowfall.

Results: Phylogenetic typing of > 400 strains from a dozen Icelandic vegetation samples revealed several candidates that are potential clones of strains from Jungfraujoch that are being further characterized and genome-sequenced to demonstrate clonality.

PW238 The personalized temporal dynamics of microbiome in the airways of cystic fibrosis patients

Giovanni Bacci[1], Giovanni Taccetti[2], Daniela Dolce[2], Federica Armanini[3], Nicola Segata[3], Francesca Di Cesare[1], Vincenzina Lucidi[4], Ersilia Fiscarelli[4], Patrizia Morelli[5], Rosaria Casciaro[5], Anna Negroni[6], Alessio Mengoni[1], Annamaria Bevivino[6]

[1]*Università degli Studi di Firenze - Dipartimento di Biologia, Sesto Fiorentino, Italy*
[2]*University Hospital Meyer, Firenze, Italy*
[3]*CIBIO -Centre for Integrative Biology, Povo, Italy*
[4]*Ospedale pediatrico Bambino Gesù, Roma, Italy*
[5]*Cystic Fibrosis Center, IRCCS G. Gaslini Institute, Department of Pediatrics, Genova, Italy*
[6]*Italian National Agency for New Technologies, Energy and Sustainable Economic Development, ENEA, Department for Sustainability, Rome, Italy*

Background: The airway of cystic fibrosis (CF) patients is a complex environment colonized by a heterogeneous mixture of microorganisms that affect patients' quality of life, disease progression, and survival. The taxonomical composition of the lung microbiota has been largely inspected although most of the conclusions drawn focus on the pathogenic signatures of the disease and still fail to give a complete picture of CF progression.

Objectives: Understand how the whole bacterial community structure changes during exacerbation events and how patients' genotype can affect these changes from both a taxonomical and a functional perspective.

Methods: Twenty-two subjects with CF, were followed over a 15-month period with attention to exacerbation events. Each subject was sampled every 3-4 months for a total of 79 samples. Functional and taxonomic features of bacterial airway microbiome of CF patients were inferred from shotgun metagenomic data obtained from sputum samples collected during medical examinations.

Results: We observed a patient-specific colonization of the main pathogens and non-classical species. Time and exacerbation events impacted the microbiome diversity, though most of the strains were still present even after clinical treatments. A core set of antibiotic resistance genes was found although their presence was not affected by antibiotic intake. In conclusion, though the lung microbiome of CF patients changed over time and along disease progression, the main CF pathogens reported a strong resilience that must be considered in future studies.

PW239 Plant growth-promoting actinobacteria associated with bryophytes

Chadabhorn Insuk[1], Wasu Pathom-aree[1]

[1]*Chiang Mai University, Biology, Chiang Mai, Thailand*

Background: Actinobacteria is a phylum of high GC Gram-positive bacteria that can produce various secondary metabolites including plant growth promoters. Studies of actinobacteria associated with plants are usually performed in higher plants. Reports from Bryophytes are very scarce.

Objectives: To isolate actinobacteria from bryophytes and investigate their plant-growth promoting activities.

Methods: Actinobacteria were isolated from 5 bryophytes, *Bryum apiculatum*, *Desmatodon gemmascens*, *Campylopus involutus*, *Frullania nepalensis* and *Plagiomnium maximoviczii* on 4 media; Water Proline Agar, R2A Agar, Humic Acid Vitamin B Agar, and Starch Casein Agar. Thirteen actinobacteria were isolated and their ability to produce indole-3-acetic acid (IAA), siderophores and to solubilize phosphate were tested using standard methods. All isolates were identified based on 16S rRNA gene sequence analysis.

Results: All isolates were able to produce IAA both with and without L-tryptophan. Isolate CMU51-1 could produce the highest amount of IAA (2.73±1.23 µg/ml) without L-tryptophan. Isolate CMU55-4 could produce the highest amount of IAA with 2 mg/ml L-tryptophan (11.35±3.34 µg/ml). For siderophore production, isolate CMU55-5 could produce the largest orange zone (1.66±0.22 cm) on Chrome Azurol S (CAS) agar. Isolate CMU51-1 could produce the highest amount of hydroxamate and catecholate types siderophore of 992.50±50.76 and 484.47±27.91µ mole/l, respectively. No isolate could solubilize phosphate. Six isolates were members of the genus *Micromonospora*. Isolate CMU55-4 shared 97.16% sequence similarity with *M. chalcea* SH2-13^{T}, suggesting a new species. Five isolates belonged to *Streptomyces* and one each of *Microbispora* and *Mycobacterium*.

PW240 Deciphering Biodiversity of Magnetotactic Bacteria in the Tropical Marine Environment of Singapore using Deep-sequencing Metagenomics

Shi Ming Tan[1], Prachi Singh[1;2], Bin Cao[1;2]

[1]Nanyang Technological University, Singapore Centre for Environmental Life Sciences Engineering, Singapore
[2]Nanyang Technological University, School of Civil and Environmental Engineering, Singapore

Background: Magnetotactic bacteria (MTB) can align and migrate along magnetic field due to the presence of magnetosomes. The known biogeographic distribution of MTB is skewed as most MTB strains have been isolated from the Northern or Southern hemisphere. Little is known about the MTB diversity in tropical, equatoral environments, where the temperature of the seabed is constant at approximately 25-28°C and the Earth's magnetic field inclination is close to horizontal.

Objectives: The objective of this study is to decipher the biodiversity of MTB in the tropical marine environment of Singapore (1°17'N 103°50'E).

Methods: Marine sediments were sampled from sites around the Straits of Singapore. Genomic DNA was extracted from the samples and deep sequencing was performed with Illumina HiSeq using the 2×250 paired end reads. Taxonomic classification of the microbial community was performed through DNA metabarcoding with the 16S rRNA gene and the conserved magnetosome gene markers.

Results: We constructed a flow cell sandwiched by two pieces of magnets where the sediment-water slurries were allowed to flow through for MTB enrichment. The 16S rRNA profiling showed that the relative abundance of MTB at one sampling site was ~0.25%, while the abundance of MTB increased to ~0.45% after the flow cell-based enrichment. Approximately, one third of the MTB could not be classified at the species level using the 16S rRNA gene analysis and the most dominant MTB in that site belong to *Magnetovibrio blakemorei*. The phylogenetic relationship among the MTB were also determined using the conserved mam gene markers.

PW241 Occurrence of Legionella spp. in reclaimed wastewater by metagenomics

Yolanda Moreno Trigos[1], Irene Hortelano Martin[1], Laura Moreno-Mesonero[1], Jorge Garcia-Hernandez[2]

[1]*Research Institute of Water Engineering and Environment, Spain*
[2]*, Biotechnology, Spain*

Background: *Legionella* is an opportunistic pathogen that mainly affects immunocompromised people or people suffering other diseases, exceptionally producing asymptomatic infections in healthy people. *Legionella spp*. are found in natural aquatic habitats and whose ecological niches are the surface waters of rivers, lakes, ponds, streams, hot springs. Currently, there are no guidelines to regulate its presence in reclaimed wastewater. Next generation sequencing (NGS) technology has been a great revolution in the pathogen detection in both clinical and environmental fields. This emerging tool allow to characterize the microbiome present in a complex sample independent of the culture and the difficulty of identification in order to assess the potential risk of exposure.

Objectives: We have investigated the occurrence of *Legionella* spp. in reclaimed wastewater used for irrigation purposes by a 16S rDNA -base metagenomics approach.

Methods: Nine samples from the tertiary treatment effluent from a wastewater treatment plant were filtered and DNA isolated by GeneJet Genomic DNA Purification Kit. V3-V4 region of 16S rRNA was amplified following Illumina protocols. Libraries were deep sequenced on a MiSeq Illumina platform using a 2×300pb paired-end run at FISABIO (Valencia, Spain). Raw DNA sequencing data was processed using QIIME™ 1.9.1 applying additional scripts available in MicrobiomeHelper virtual box.
Results: Metagenomics analysis showed that 7 of the analyzed samples were positive for *Legionella spp*. including *L. pneumophila.* The results show the role of reused water as a reservoir and potential infection source for *Legionella spp.*

with grants for **first projects (PAID-06-18) by The Polytechnic University of Valencia (UPV)**

PW242 Is there a relationship between outer membrane vesicles (OMVs), ATP content and biofilms?

Nicolas Baeza[1], Elena Mercade[1]

[1]*University of Barcelona, Biology, Healthcare and Enviroment, Barcelona, Spain*

Background: OMVs are ubiquitous in the extracellular matter of many gram-negative bacteria, and for some them their production has been involved in biofilm formation. OMVs are known to carry inside different elements, such as adenosine triphosphate (ATP) an extremely valuable storage molecule to all cells. Release of extracellular ATP (eATP) by growing bacterial cells has been reported, but its delivery inside OMVs and its correlation with biofilm formation remain unexplored.

Objectives: To determine if there is a correlation between the ability of gram-negative Antarctic bacteria to form biofilms, their production of OMVs and their contain in ATP.

Methods: OMV were isolated by high-speed centrifugation and characterized by transmission electron microscopy (TEM). OMVs concentration was measured by their LPS content using the Purpald assay and their protein content. ATP levels were measured by bioluminescence. Determination of biofilm formation was determined by the crystal violet method and confocal microscopy.

Results: We determined the presence and amount of OMVs in a collection of Antarctic strains from our laboratory. We found the presence of eATP in the cultures of many of them and we confirmed for *Shewanella vesiculosa* M7^T that most of eATP was contained inside the OMVs (70-80%). Some of the Antarctic strains produced biofilms but globally this ability was not correlated with the presence of OMVs neither with the secretion of eATP. Exogenously provided OMVs as well as exogenously provided ATP increased biofilm formation in *S. vesiculosa* M7^T cultures.

PW243 Removal of fuel oil by bacterial consortium formulated from effective hydrocarbon-degrading bacteria

Duangporn Polrit[1], Chanokporn Muangchinda[2], Onruthai Pinyakong[2]

[1]Chulalongkorn University, International Program in Hazardous Substance and Environmental Management, Graduate School, Bangkok, Thailand
[2]Chulalongkorn University, Faculty of Science, Department of Microbiology, Bangkok, Thailand

Background: Petroleum hydrocarbon contamination in freshwater caused by oil spill and water transportation is considered as important environmental problem. The petroleum oil used in cargo ship is fuel oil which is the complex mixtures of polycyclic aromatic hydrocarbons (PAHs), aliphatic compounds, asphaltene and resin.

Objectives: This study aims at formulation of the bacterial consortium by co-cultivation of the selected effective hydrocarbon-degrading bacteria for degradation of petroleum oils.

Methods: Three bacteria having different ability to degrade various types of PAHs and aliphatics were selected among several hydrocarbon-degrading bacteria isolated from river sediment samples. The degradation efficacy of fuel oil by the consortium in form of free cells and immobilized cells on plastic ball was examined.

Results: *Mycolicibacterium* sp. J101 was capable of degrading low- and high-molecular weight PAHs and aliphatic compounds. *Rhodococcus ruber* S103 degraded aliphatic compounds and had high cell surface hydrophobicity. *Mycolicibacterium* sp. Y502 degraded high-molecular weight PAHs and had potential to produce biosurfactant. The consortium containing these bacteria degraded 41% of 3,000 mg L^{-1} fuel oil within 7 days. While, the individual strains degraded only 10 – 20%. The consortium also degraded 100% of diesel oil (2,000 mg L^{-1}), pyrene (100 mg L^{-1}) and docosane (100 mg L^{-1}) within 3, 3 and 7 days, respectively. Furthermore, the immobilized consortium could remove 50% of fuel oil from cultivation medium based on both adsorption and biodegradation abilities. The same trend was obtained in freshwater system. The results suggested the potential use of the immobilized bacterial consortium for bioremediation of oil-contaminated environments.

PW244 Employing synthetic bacterial communities to prevent plant invasion by Ralstonia solanacearum

Alicia Jimenez Fernandez[1], Stijn Spaepen[1], Yuji Ishigaki[2], Paul Schulze-Lefert[1]

[1]*Max Planck Institute for Plant Breeding Research, Köln, Germany*
[2]*RIKEN Center for Sustainable Resource Science, Yokohama, Japan*

Background: The *Ralstonia solanacearum* species complex is a group of pathogenic bacteria that infects over 200 host plant species worldwide, including the model plant *Arabidopsis thaliana,* and causes significant economic losses. Bacteria infect the plant through the roots and spread systemically via the vasculature, blocking water transport and resulting in wilt disease and eventual death. To reach and colonize its host, *R. solanacearum* must survive in the rhizosphere microbiome, a multispecies microbial community associated with plant roots. Previous research shows that the microbiome confers plant protection against *R. solanacearum* in a host genotype-dependent manner, but these interactions are not well understood.

Objectives: We aim to understand how *R. solanacearum* invades taxonomically diverse bacterial communities associated with *A. thaliana* and to elucidate the impact of these communities on pathogen root colonization.

Methods: We characterized binary interactions between a 194-isolate collection of *A. thaliana* root-associated bacteria and *R. solanacearum* GMI1000. Subsequently, adopting a high-throughput automated millifluidic system, we tested the growth of a GFP-tagged *R. solanacearum* derived from GMI1000 in standing and nascent taxonomically diverse synthetic communities (SynComs) composed of members of our bacterial collection.

Results: We have identified several strains from the taxonomically diverse genera *Pseudomonas, Streptomyces, Bacillus*, *Arthrobacter* and *Achromobacter* that exert antagonistic activity against *R. solanacearum*. We have also identified potential minimal SynComs that inhibit *in vitro* growth of *R. solanacearum*. We now plan to test their potential as biological control agents in *A. thaliana* flow pot systems.

PW245 Microbiota in the low-latitude regions of the Pacific Ocean functionally discriminated by metagenomics

Hideto Takami[1], Yuya Tada[2], Wataru Arai[2], Takashi Okubo[2], Yoshitoshi Ogura[3], Tetsuya Hayashi[4], Shotaro Suzuki[5], Koji Hamasaki[5]

[1]Japan Agency for Marine-Earth Science and Technology, Yokohama Institute, Yokohama, Japan
[1]Japan Agency for Marine-Earth Science and Technology, Yokohama Institute, Yokohama, Japan
[3]Kyushu University, Bacteriology, Fukuoka, Japan
[4]Kyushu University, Biotechnology, Fukuoka, Japan
[5]The University of Tokyo, Atmosphere and Ocean Research Institute, Kashiwa, Japan

Background: Although one of the main goals of metagenomics is the actual deduction of not only species diversity but also potential comprehensive functions harbored by entire communities, diversity estimates based on 16S rDNA has just been discussed in most studies. Also, whereas the genetic composition of marine microbial communities varies at the microscale between particle-associated (PA) and free-living (FL) niches, it remains unclear how metabolic potentials differ between them.

Objectives: The aim of this research is to elucidate the difference in metabolic and physiological potential of microbial consortia between subtropical and tropical regions of the Pacific Ocean and also between PA and FL fractions.

Methods: Totally 18 seawater samples were collected from the surface at 9 sites in the 3 Pacific Ocean areas of North and South Subtropical Gyre, and an eastern equatorial region. These samples were filtered to separate PA and FL fractions. DNAs extracted from each sample were sequenced by MiSeq and the fastq files were treated to generate multi-FASTA file of amino acid. These files were applied to MAPLE system for functional analysis.

Results: There were significant differences in the abundance of functional KEGG modules between PA and FL fractions. In addition, significant differences in module abundance in PA or FL fraction among 3 ocean areas were shown except for one equatorial site. Thus, our results indicate that it is possible to define ocean sector based on potential abundance of the metabolic and physiological functions as these results were also supported by the statistical analysis (NMDS).

PW246 Metagenomic study on the microbial communities of deteriorated medieval frescoes

Marina Fomina[1], Ji Hong[2]

[1]*Institute of Microbiology and Virology of National Academy of Sciences of Ukraine, Department of Industrial Microorganisms Physiology, Kyiv (=Kiev), Ukraine*
[2]*Marine Biodiversity Institute of Korea, Department of Taxonomy and Systematics, Korea, Rep. of South*

Background: Both eukaryotic and prokaryotic microorganisms can play a key role in the deterioration of the historic and cultural monuments and artifacts. Recently, the unique ancient frescoes in the 11th c. cathedral have been suffered from dark-spotted deterioration.

Objectives: The aim of this work was to elucidate the nature of frescoes deterioration by using the metagenomic assessment of microbial communities that might be involved in the dark spots formation.

Methods: The culture independent PCR-based molecular biological approach involved the cloning of the ITS amplicons for microfungi and 16S rDNA for bacteria.

Results: Metagenomic analysis of the fungal DNA revealed that xerophilic aspergilli play an important role in the dark-spotted deterioration of frescoes demonstrating the dominance of *Eurotium halophilicum* as monoculture or in the association with *Aspergillus vitricola*, with the latter not exceeding 33% of fungal clones. It was also found the prevalence of the spore-forming and common in indoor air, dust and soil environments Firmicutes bacteria in the sites of deteriorated frescoes. However, considering the absence of significant difference between the bacterial communities in the deterioration sites and control undamaged areas, bacteria are unlikely to play a major role in the development of dark spots on frescoes. Further detailed and comprehensive study of the biological and abiotic mechanisms of the frescoes deterioration is needed for better understanding how to stop microbial invasion and deal with its consequences.

PW247 Dust-borne viruses may negatively affect Prochlorococcus biomass

Eyal Rahav[1], Adina Paytan[2], Esra Mescioglu[2], Edo Bar-Zeev[3], Barak Herut[1]

[1]*Israel Oceanographic and Limnological Research*
[2]*University of California Santa Cruz, Santa Cruz, United States*
[3]*Ben Gurion University of the Negev, Israel*

Background: Recent studies have demonstrated that viable airborne microbes (fungi, prokaryotes, viruses) may affect oceanic production and microbial biomass upon deposition in surface seawater.

Objectives: This study explores the novel role of aerosols-derived viruses in diminishing the abundance of the ubiquities cyanobacteria *Prochlorococcus* in marine systems subjected to relatively high atmospheric dust inputs.

Methods: Aerosol enrichment microcosms were carried where 'UV-killed dust' (leached nutrients/ trace-metals) or 'live-dust' (leached nutrients/trace-metals and airborne microbes) samples were added to seawater or *Prochlorococcus* MED4 cultures, and changed in *Prochlorococcus* biomass was recorded. Next, *Prochlorococcus* leachate (dissolved fraction which includes the <0.2-µm particles such as viruses) was transferred to a fresh culture and *Prochlorococcus* biomass was recorded again. Transmission-electron-microscopy (TEM) images were taken to examine *Prochlorococcus*-infected cells.

Results: We show that *Prochlorococcus* cells can be infected by airborne viruses, resulting in low relative abundance in the Mediterranean surface water. Bacteriophages-infected *Prochlorococcus* MED4 were observed using TEM only in cells cultured with 'live-dust' and not in those treated with a UV-sterilized aerosol. Amendments of dust particles or dust-leachate (<0.2-µm) to seawater or *Prochlorococcus* cultures resulted in a significant decline in cell density (<90%), while UV-sterilized dust elicited a much weaker response. We suggested that the dominance of *Synechococcus* over *Prochlorococcus* throughout the surface Mediterranean-Sea and the typical spatial trends in chlorophyll-*a* are regulated by airborne viral lysis of *Prochlorococcus*. Predictions for enhanced desertification and larger amounts of mineral aerosol in the atmosphere may intensify the transport of airborne viruses to marine systems.

PW248 Enrichment and characterization of microorganisms capable of degrading various C1 compounds in the Black Sea

Peter Fischer[1;2], Alfons J.M. Stams[1;3], Laura Villanueva[2], Diana Sousa[1]

[1]Wageningen University & Research, Laboratory of Microbiology, Wageningen, Netherlands
[2]Royal Netherlands Institute for Sea Research, Department of Marine Microbiology & Biochemistry, 't Hoorntje, Netherlands
[3]University of Minho, Centre of Biological Engineering, Braga, Portugal

Background: Methylated compounds can be used as an energy source to drive interactions between sulfate reducing microorganisms and methanogens. This has potential impact on the current understanding of the global carbon and sulfur cycles.

Objectives: The use of methylated compounds by anaerobic microorganisms present in the sulfidic permanently stratified Black Sea sediment and column water and the composition of these communities was investigated through enrichment studies.

Methods: Black Sea sediment of three different depths between 5 and 30 centimeters below sea floor, as well as water at 105 meters deep were collected anoxically and used for enrichments, supplemented with 1 mM of either dimethylsulfide (DMS), dimethylsulfoniopropionate (DMSP), trimethylamine (TMA) and methanol as sole energy source. To promote methanogenesis, acetogenesis and sulfate reduction in the different enrichments, 20 mM molybdate, 20 mM bromoerhanosulfonate (BrES) and 20 mM BrES with 20 mM sulfate was added, respectively. Anoxic cultures were incubated at 20^{o}C in the dark. Uptake of substrate and product formation were monitored over 4 weeks. Active cultures were transferred to fresh medium to promote further enrichment. Analyses of 16s rRNA gene sequencing are ongoing to elucidate the inocula and culture communities.

Results: All enrichments grew on the provided substrates. Over four weeks, utilization of substrate ranged between 20% and 100% for all enrichments. Subsequent transfers of the enrichments retained the decrease of substrate although utilization was slower. These results will be complemented with 16s rRNA gene sequencing data and community comparison developed n methanogenic, acetogenic and sulfate-reducing enrichments performed.

PW249 Denitrification and Resistome of Sediment Communities Impacted by Wastewater Discharge of a Poultry Processing Plant

Miguel Semedo[1], Bongkeun Song[1]

[1]*Virginia Institute of Marine Science, Biological Sciences, Gloucester Point, United States*

Background: Microbial communities in tidal creek sediments provide an important ecosystem service of removing excess nitrogen (N) through denitrification. This process can be impaired by chemical pollutants, such as antibiotics, released from Concentrated Animal Feeding Operations (CAFOs) or animal processing plants. Due to the use of selected antibiotics in CAFOs, a unique resistome is expected to be found in impaired tidal creeks.

Objectives:

1) Determine the impacts of wastewater discharge from a poultry processing plant on microbial denitrification and resistome structure in tidal creek sediments.

2) Investigate the relationships between denitrification and the resistome of microbial communities.

Methods: We conducted a seasonal survey in two tidal creeks (impacted vs. reference) in a region where poultry industrial farms are abundant. The "impacted" creek has received direct discharges of treated wastewater from a poultry processing plant, while the "reference" creek has no wastewater discharge. Sediments were sampled at three locations along salinity gradients in both creeks. Denitrification activities were measured using slurry incubations with $^{15}NO_3^-$. Shotgun metagenome analysis was conducted to examine the abundance and diversity of denitrifiers and the resistome in the sediment communities.

Results: Denitrification rates were generally lower in the impacted creek than the reference creek. The abundance of denitrification genes was also lower near the discharging site where a unique resistome was found. Our results indicate that tidal creek microbial communities and associated denitrification activities were disturbed by the exposure to processing plant wastewater. These impacts were partially explained by links between antibiotic resistance and denitrification potential.

PW250 High Rate of Gut Colonization by CTX-M-15 type ESBL Producing Enterobacteriaceae in Subsistence Farming Communities of Rural Nepal: a One Health Approach

Supram Hosuru Subramanya[1], Indira Bairy[2], Niranjan Nayak[1]

[1]*Manipal College of Medical Sciences (MCOMS), Pokhara, Nepal*
[2]*Melaka Manipal Medical College, Manipal, India*

Background: Increasing trend of gut colonization by extended-spectrum β-lactamase producing Enterobacteriaceae (ESBL-E) was observed in commercial farming animals and their owners, but such colonization among domesticated livestock has not been well studied.

Objectives: To determine the gut colonization rate of ESBL-E and carbapenemase-producing Enterobacteriaceae (CPE) among subsistence farming communities of rural Nepal.

Methods: Altogether 452 rectal swabs and 127 sewage sample were collected by systematic random sampling, from 127 farming community houses in Kaski district of Nepal. Organic feed pasture-raised livestock and their owners without history of antibiotic consumption or hospitalization during the last 6 months were included in the study. Specimens were screened for ESBL-E and CPE by selective plating and confirmed by phenotypic methods. The β-lactamase genes; blaTEM/blaSHV/blaOXA-1 and blaCTX-M (phylogenetic groups 1, 2 and 9) were detected by PCR assays.

Results: A total of 358 (61.8%, 95% CI 60, 68) ESBL-E isolates were obtained from 579 specimens, which included 95 ESBL-E (74.8%) from 127 adult humans, 100(78.74%) from 127 of their children, 56 (56.6%) from 99 cattle, 24(51.1%) from 47 goats, 19 (36.5%) from 52 poultry and 64 (50.4%) from 127 environmental samples. No CPE was isolated from any of the samples. blaCTX-M-15 was the most predominant genotype found both in animal (74.8%) and human (70.4%) isolates. More than 50% of the isolates were MDR. The higher number of animal isolates was resistant to ciprofloxacin, nitrofurantoin, and amikacin. This foremost study in Nepal demonstrating ESBL gene reservoirs in subsistence farming communities is a serious public health concern.

PW251 Low bacterial diversity and the presence of antimicrobial residues and antimicrobial resistance genes in a river containing wastewater from backyard aquacultures in the Mekong Delta, Vietnam

Tatsuya Nakayama[1], Hoa Tran Thi Tuyet[2], Kazuo Harada[3]

[1]*National Insititute of Health Scinece, Division of Biomedical Food Research, Japan*
[2]*Can Tho University, College of Aquaculture and Fisheries, Viet Nam*
[3]*Osaka University, Faculty of Medicine*

Background: Environmental pathways of antibiotic resistance have received attention. Water environments act as reservoirs or sources of antimicrobial-resistant bacteria (ARB), antimicrobial residue, and antimicrobial resistance gene (ARG).

Objectives: The aim of this study is to identify the role of polluted water in dissemination of antimicrobial resistance.

Methods: Twelve freshwater sites were selected from among the aquacultures and rivers in Can Tho, Vietnam and were analyzed for antimicrobial residues, ARGs and water microbiota by LC/MS/MS, real-time PCR, and 16S rDNA-based metagenomic analysis. Moreover, the presence of extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* was investigated from the gut contents of 54 freshwater fish.

Results: Residues of sulfamethoxazole (SMX) (10/12) and sulfadimidine (SDD) (7/12) were widely detected, together with the *sul1* gene (11/12). Additionally, both SMX and the $bla_{CTX\text{-}M\text{-}1}$ gene mostly originated from human activity were detected in freshwater systems (8/12). Metagenomic analysis showed that all the evaluated sites contained *Proteobacteria*, *Actinobacteria*, and *Bacteroidetes*, representing 64% of the total microbiota. Moreover, *Proteobacteria* comprised more than 90% of the total microbiota in the river site of (Ri-E), located at the merging point of wastewaters from backyard aquacultures. Bacterial diversity analysis showed that Ri-E exhibited the lowest diversity. Moreover, cultured striped catfish had more ESBL-producing *E. coli* than other fish. In conclusion, sulfa drug residues and ARGs are widely detected. The river site Ri-E had remarkably low bacterial diversity, which was probably influenced by the wastewater from backyard aquaculture. More than 38% of freshwater fish had ESBL-producing *E. coli*.

PW252 Mechanisms of bacterial short-term co-adaption

Nathalie Nina Suhr Eiris Henriksen[1], Jakob Herschend[1], Mads Frederik Hansen[1], Mette Burmølle[1]

[1]*Section of Microbiology, Department of Biology, University of Copenhagen, København, Denmark*

Background: Bacteria live in complex and co-evolved communities, which are formed by the presence of other bacteria and their interspecies interactions. The interspecies interactions lead to emergent properties that cannot be identified through analysis of the individual parts. Currently, research interests are directed towards studies of complex communities, why it is important to improve our understanding of the mechanisms enabling coexistence and specifically the interspecies interactions driving co-adaptation and co-evolution.

Objectives:

- Design of a short-term experimental evolution experiment selecting for biofilm producers, thus facilitating co-adaptation.
- Develop variants that display changed phenotypic behavior compared to ancestral strains.
- Characterize interspecies interactions in short-term co-cultivation.
- Compare the spatial organization between evolved and ancestral co-cultures.

Methods: Co-cultures of *Lactococcus lactis ssp. cremoris* and *Leuconostoc mesenteroides ssp. mesenteroides* are used in a short-term evolution experiment where transfer of biofilm associated cells adhered to steel beads acts as the selective force. Evolved and ancestral strains are assessed in different assays to identify changed phenotypic behavior, and whole genome sequencing is subsequently performed on selected variants, elucidating underlying genetic modifications. Fluorescent marker gene technology and confocal laser scanning microscopy is used to visualize the spatial organization of the co-cultures and proteomics to expose interspecies interactions.

Results: Evolved variants display longer generation time compared to ancestral strains. This indicates one or several potential trade-offs between growth and other parameters likely to be associated to attachment abilities and biofilm formation. Our experimental design selected for variants which requires further examination to elucidate mechanisms of co-adaptation.

PW253 Deciphering strategies of a river-sediment microbial community to cope with anthropogenic metal contamination

Valentine Cyriaque[1], Jacquiod Samuel[2], Leise Riber[3], Waleed Abu Al-Soud[4], Gillan David[1], Søren Johannes Sørensen[4], Ruddy Wattiez[1]

[1]*UMONS, Proteomics and Microbiology, Mons, Belgium*
[2]*INRA Centre Dijon, UMR1347 AGROECOLOGIE, Dijon, France*
[3]*University of Copenhagen, Section for Functional Genomics, Copenhagen, Denmark*
[4] *University of Copenhagen, Section of Microbiology, Copenhagen, Denmark*

Background: Metal contamination poses biotoxicity and bioaccumulation issues, affecting both abiotic conditions and biological activity. For a century, the MetalEurop foundry released zinc, copper, cadmium and lead directly into the river “la Deûle”, resulting in a 30-fold increase in metal concentrations in downstream sediments compared with upstream sediments.

Objectives: We applied an integrative approach coupling *in situ* sequencing of both DNA and RNA and Horizontal Gene Transfer (HGT) analysis in order to understand the mechanisms driving community adaptation to metal pollution.

Methods: We investigated the structures of both total and active fractions of sediment prokaryotic microbiomes by coupling DNA and RNA-based sequencing. We applied the ecological concept of Functional Response Groups (FRGs) to decipher the adaptive tolerance range of the sediment communities through characterization of microbial strategists. Deeper HGT analysis using *in vitro* conjugation assays coupled to Fluorescence Activated Cell sorting (FACS) and 16S rDNA sequencing to explore the long-term metal impact on plasmid permissiveness of the community.

Results: Obtained results revealed the presence of an enriched native pool of conjugative plasmids in the polluted sediments, highlighting their importance for long-term adaptation of the community facing metal contamination, being in line with our *in situ* observation. Altogether, this integrative study reinforces the notion that microbial strategies were established to face the metal pollution, such as facilitation processes, microbial community coalescence and HGT, leading to an unexpected higher microbial diversity.

PW254 Implementation of Biological Reactor for Biosynthesis of Iron oxide minerals with Arsenic Removal Properties using an Acidophilic Fe(II)-oxidizing bacterial Consortium As-resistant, isolated from Atacama Desert, Chile.

Carla Geraldine León Lemus[1], Matias Castro[1], Jorge Durango[2], Javiera Ravanal[1], Cristian Valenzuela[1]

[1]*University of Concepción, Department of Microbiology, Concepcion, Chile*
[2]*University of Concepción, Department of Materials Engineering, Concepcion, Chile*

Background: Sloman reservoir is an Arsenic (As) contaminated area located at the Loa River ubicated in the Antofagasta region, Chile. Arsenic is leached out of volcanic materials of the Andes, resulting in high concentrations of As (0.1–1.5 mg L-1) in sediments and waters, representing a serious health concern. Among the treatment for As-removal, adsorption is considered to be the most promising technology. Iron oxide minerals present high sorption capacity and strong As-binding affinity.

Objectives: The present study aimed to develop a batch bioreactor for the synthesis of iron oxide minerals using an acidophilic Fe(II)-oxidizing bacterial consortium As-resistant, isolated from a highly As contaminated environment.

Methods: Acidophilic Fe(II)-oxidizing bacterial consortium (CL5) As-resistant able to bio-synthesized iron oxide minerals was isolated from a sediment sample from Sloman reservoir and characterized microbiologically. The obtained bio-minerals were characterized by XRD and SEM-EDX.

Results: Result showed that CL5 Consortium tolerated high concentration of As(III) (64 mM) and As(V) (>350 mM) and low pH (up to 1.5). Fe(II)-oxidation was optimum at an initial pH of 2.5 and temperature of 28°C. XRD patterns showed dominance of amorphous minerals with peaks described for schwertmannite and jarosite, while SEM-EDX analysis showed presence of Fe-S-As compounds in precipitates, indicating an interaction with As by sorption. These results indicate that acidophilic/metal-tolerant bacterial culture serve as a simple system for the synthesis of iron oxide minerals with environmental application in arsenic removal from As-bearing waters.

PW255 How does cold atmospheric pressure plasma (CAPP) impact bacterial biofilms formed on hospital surfaces?

Sarah Kennedy[1], Muireann Fallon[1], Stephen Daniels[2], Hilary Humphreys[1;3]

[1]Royal College of Surgeons in Ireland, Department of Clinical Microbiology, Ireland
[2]Dublin City University, National Centre for Plasma Science and Technology, Ireland
[3]Department of Clinical Microbiology, Beaumont Hospital, Ireland

Background: Healthcare associated infections affect patients around the world. More effective methods of decontamination are evaluated to protect patients given shortcoming in existing approaches such as the potential toxicity of chemical agents.

Objectives: Our laboratory has demonstrated that cold atmospheric pressure plasma (CAPP) has bactericidal and sporicidal activity. The anti-microbial effects of CAPP are due to a cocktail of different reactive species including ultraviolet radiation, reactive oxygen and nitrogen species and charged particles. Here we evaluate the effects of CAPP on bacterial biofilms formed by *Escherichia coli*, and methicillin resistant *Staphylococcus aureus* (MRSA).

Methods: Biofilms were formed on various surfaces found in clinical wards such as stainless steel, powder coated steel, glass etc. They were treated with CAPP and analysed using traditional crystal violet assays to determine effects on biomass, the resazurin assay to determine viability, confocal laser scanning microscopy (CLSM) to assess the ratio of live and dead cells and scanning electron microscopy (SEM) to examine the morphology of the cells present after treatment.

Results: The anti-microbial effects of CAPP on biofilm biomass were species and surface specific. However, CAPP treatments lead to an increase in dead cells within the bacterial biofilms analysed by confocal microscopy and affected the numbers of cells present and morphology of cells observed by SEM compared to the untreated controls. Initial experiments indicate that CAPP has activity against biofilm on surfaces commonly found in acute hospitals. However, further research is required to confirm effectiveness in the clinical setting, with other pathogens, and different conditions.

PW256 Radiation Resistance in the Cyanobacterium Arthrospira

Anu Yadav[1;2], Natalie Leys[1], Pieter Monsieurs[1;3], Ann Cuypers[2], Paul Janssen[1]

[1]*SCK.CEN, Mol, Belgium*
[2]*Universiteit Hasselt, Hasselt, Belgium*
[3]*Vlaamse Instelling voor Technologisch Onderzoek (VITO), Mol, Belgium*

Background: The multicellular cyanobacterium *Arthrospira* has been studied for many years because of its excellent nutritive value as a food- and feedstock and its many applications in biomedical sciences. We study *Arthrospira* sp. PCC 8005 as a principal organism and edible endproduct of the MELiSSA bioreactor, a life support system being developed by the ESA.

Objectives: Our aim is to understand the genetic and biochemical pathways involved in the resistance of strain PCC 8005 to high doses of Co-60 gamma radiation (GR).

Methods: We analyzed strain PCC 8005 before and after acute and chronic exposure to GR for culture-based cell recovery, morphological changes, variations in cellular contents of proteins, pigments, and carbohydrates, genome sequencing, and transcriptomics.

Results: During continuous cultivation of strain *Arthrospira* sp. PCC 8005 under controlled conditions we observed that slight variations in growth conditions can have a profound and permanent effect on its trichome morphology (spiral to straight). We noted differences between these morphotypes in the amount of light harvesting antenna, the cellular content of hydrophilic and lipophilic proteins, trehalose and glutathione content, and GR resistance. The two morphotypes displayed 57 genetic differences (10 indels, 43 SNP's and 4 large insertions) adversely affecting a total of 14 genes. Only 4 of these genes have a known function (*purA, intA*, *gvpC* and *psbD1*) but they have no apparrant association with the observed switch in morphotype so we are now extending our analyses with RNAseq transcriptomics to elucidate whether any non-coding RNA's are at play.

PW257 Creation of a preliminary inventory of bacteria associated to minery affected soil in Buenos Aires, Cauca

Patricia Velez[1], Ivon Bolaños[1], Daniela Quibano[1], Alexis Sanchez[1], Christian Franco[1], Neyl Sanchez[1], Daniel Velasco[1], Beatriz Adrada[1], Diego Zuñiga[1], Javier Rosero[1], Melissa Tobar[1], Erika Camacho[1], Irene Urbano[1], Laura Carvajal[1], Yuleissi Tandoy[1], Marisol Estupiñan[1], Greace Zambrano[1], Alejandra Campo[1]

[1]*Universidad del Cauca, FACNED, Departamento de Biología, Popayán, Colombia*

Background: In Colombia mining is one of the oldest economic activities, it represents a high percent of environment contamination in the ecosystems where these practices are developed. Some bacteria that grow in these contaminated soils have enzymes which are able to metabolize pollutants and stimulate bioremediation processes.

Objectives: Due to the fact that the bacterial species in soil affected by mining in the department of Cauca has not been studied, a collection of bacteria with potential use in bioprospective processes in the region was proposed,

Methods: to aim this objective we made a preliminary identification of bacteria associated with four neighboring points of the "La Puchis" mine in Buenos Aires, Cauca, now Sociedad Minera Sur. Through process like isolation and macro and microscopic identification of bacteria

Results: we achieve our objective and could establish a preliminary inventory of bacteria associated to mine soils with a potential use in bioprospective processes on the region. The findings offer possibilities for the realization of later studies to favor ecosystems disturbed or impacted by anthropic activities such as mining. This study is a pioneer for the analysis of the environmental impact and potential solutions in the Departments affected by mining in Colombia.

PW258 Antimicrobial Mechanisms of Plasma Treated Liquid

Muireann Fallon[1], Sarah Kennedy[1], Stephen Daniels[2], Hilary Humphreys[1;3]

[1]*Royal College of Surgeons in Ireland, Department of Clinical Microbiology, Ireland*
[2]*Dublin City University, National Centre for Plasma Science and Technology, Ireland*
[3]*Beaumont Hospital, Department of Microbiology, Ireland*

Background: Healthcare-acquired infections (HCAIs) are a growing concern the world over. Recent research and a greater understanding of cold atmospheric pressure plasma (CAPP) systems suggests that this technology may have a role in hospital decontamination and in preventing the spread of HCAIs. CAPP can not only directly inactivate bacteria, but can also be used indirectly through plasma activated liquids (PAL). The mechanisms of action of both CAPP and PAL are not yet fully understood.

Objectives: To examine the possible mechanisms of antimicrobial action of CAPP treated phosphate buffered saline (PBS).

Methods: PBS was treated with CAPP for 90 or 300 seconds. *Staphylococcus* and *Escherichia* were inoculated with the PAL overnight and resulting viability was assessed through CFU assays. PAL was then examined for levels of reactive oxygen and reactive nitrogen species (RONS) using colourmetric assays. *Staphylococcus aureus* and *Escherichia coli* were inoculated in PBS with similar levels of RONS and viability was also assessed through CFU assays.

Results: Planktonic *Staphylococcus aureus* and *Escherichia coli* were successfully inactivated after inoculation overnight in PAL. The levels of RONS present in PAL increased with CAPP treated time of the liquid which in turn corresponded to increased bacterial death. PBS with bacteria and with similar levels of reactive species to that of the PAL produced similar microbial inactivation. The RONS produced by the CAPP treatment of liquid could be responsible at least in part for the bacterial inactivation.

PW259 Insights on nitrate reduction by Geobacter sulfurreducens

AURORA M PAT-ESPADAS[1], Alberto Hernandez Eligio[2], Katy Juarez[3], francisco j cervantes[4]

[1]*CONACYT-UNAM INSTITUTO DE GEOLOGIA, Mexico*
[2]*CONACYT-INSTITUTO DE BIOTECNOLOGIA UNAM, Mexico*
[3]*INSTITUTO DE BIOTECNOLOGIA UNAM, Mexico*
[4]*INSTITUTO POTOSINO DE INVESTIGACION CIENTIFICA Y TECNOLOGICA A.C, Mexico*

Background: Geobacter sulfurreducens has an exceptional metabolic capacity that can be used for contaminants removal by coupling the oxidation of acetate or hydrogen to the reduction of several terminal electron acceptors. In this study, it was explored the capacity of G. sulfurreducens to reduce nitrate in the presence and in the absence of biogenic palladium nanoparticles (Pd NPs), which has not been reported so far.

Objectives: Evaluate the capacity of G. sulfurreducens to reduce nitrate with acetate and hydrogen as electron donors and in the presence of biogenic Pd NPs to investigate catalitic denitrification.

Methods: Pure culture of Geobacter sulfurreducens strain PCA was routinely cultured, harvested and exposed to concentrations of 100 mg NO_3^-/L. Biogenic Pd NPs were synthesized as described in Pat-Espadas, et al., 2013 and added to selected experiments. Control assays were carried out in cell-free media. All assays were incubated at 28° C and performed in duplicate. Samples were analyzed at specific time intervals to quantify N-NH_4^+, N-NO_3^- and N-NO_2^-; RT-qPCR analysis were also carried out.

Results: The results obtained from this study demonstrated that G. sulfurreducens is able to reduce nitrate using acetate as electron donor after overcoming a lag phase of 100 h. Genes nrfH y nrfA were overexpressed under these conditions. The maximum rates of nitrate conversion, when Pd NPs were added to the medium, were 1.6 and 2 times higher as compared to the control without Pd NPs when using acetate (1.08 mg NO_3^-/L*h) and hydrogen (2.36 mg NO_3^-/L*h) as electron donors, respectively.

PW260 Treatment of contaminated soil mesocosms by autochthonous selected bacterial and fungal consortia

Giulia Spini[1], Federica Spina[2], Anna Poli[2], Ilaria Re[3], Carla Gramellini[4], Cristina Menta[5], Giovanna Cristina Varese[2], Edoardo Puglisi[1]

[1]Università Cattolica del Sacro Cuore, Department for Sustainable Food Processes (DiSTAS), Piacenza, Italy
[2]Mycotheca Universitatis Taurinensis- Università di Torino, Department of Life Sciences and Systems Biology, Torino, Italy
[3]Consorzio Italbiotec, Milano, Italy
[4]Arpae Emilia Romagna, Bologna, Italy
[5]Università di Parma, Department of Chemistry, Life Sciences and Environmental Sustainability, Parma, Italy

Background: The EU-project LIFEBIOREST aims to bioremediate a soil highly contaminated by polycyclic aromatic hydrocarbons (PAHs), volatile compounds (BTEX) and alkanes exploiting selected consortia of autochthonous fungi and bacteria.

Objectives: In this perspective, fungi and bacteria isolated from contaminated soil by enrichment procedure as being most effective in degrading target pollutants were tested in mesocosms biodegradation experiments.

Methods: Specifically, mesocosms were made up of 15 Kg of contaminated soil treated with six consortia (namely A-B-C-D-E-F) of fungal and bacterial isolates to identify consortia that give the best degrading performances. The selection of these consortia was made considering the pollutants degradation efficiencies assessed by massive microcosms screening and biosurfactants production. The treated and control mesocosms were maintained under controlled conditions, and sampled at 0, 20, 60 and 90 days for microbiological, chemical and ecotoxicological monitoring.

Results: Chemical analyses addressed the total content of main site contaminants: PAHs, BTEX, alkanes (C>12). Microbial consortium A showed the best performance: a concentration reduction in time for all the contaminants analyzed and a remarkable degradation towards heavy hydrocarbons (C>12) and 4-rings PAHs. Ecotoxicological results (germination test, *Daphnia mania*, *Vibrio fischeri*, *Eisenia fetida*, *Folsomia Candida*) at final time compared to zero time pointed out a response heterogeneity with a general breakdown of toxicity associated with the biological treatment.

For each mesocosm, DNA was also extracted, amplified for bacterial (16S) and fungal (ITS) biomarkers, and the resulting amplicons sequenced in Illumina. Results will be presented with the aim of assessing the ecological evolution of the microbial communities.

PW261 Pollution-induced community tolerance in soil bacteria exposed to oxytetracycline

Maddi Artamendi[1;2], Leire Jauregi[1], Lur Epelde[1], Itziar Alkorta[3], Carlos Garbisu[1]

[1]*NEIKER-Tecnalia, Conservation of Natural Resources, Soil Microbial Ecology Group, Derio, Spain*
[2]*University of Basque Country (UPV/EHU), Bilbao, Spain*
[3]*Instituto BIOFISIKA (CSIC, UPV/EHU), Biochemistry and Molecular Biology, Bilbao, Spain*

Background: The application of animal manure as fertilizer into soil can enhance soil physicochemical and biological properties. This agricultural practice can lead to contingent risks associated with the incorporation of traditional and emerging contaminants into soil. Among the emerging contaminants, antibiotics and their residues, antibiotic resistance genes, antibiotic resistant bacteria and mobile genetic elements are of particular interest due to the current situation of increasing emergence and dissemination of antimicrobial resistance. This work was funded by the Basque Government through URAGAN-CRN 18-20 (18-00044).

Objectives: Pollution-induced community tolerance (PICT) is a parameter of great value to establish a causal relationship between the presence of a contaminant and the response of a biological community. The main objective of this study was to investigate pollution-induced community tolerance in soil bacterial communities exposed to oxytetracycline (OTC).

Methods: Soil was amended with cow manure, from an ecologically certified farm, that had previously been spiked with three doses (normal, high and extremely high) of OTC. After an exposure time of 21 days, PICT to OTC was determined in soil bacterial communities exposed to 10 concentration of OTC using Biolog EcoPlates™, according to several parameters: concentration that reduced the colour formation to 50% of the maximum colouring (EC_{50} values), area under the curve, slope, Shannon's diversity and Pielou´s evenness.

Results: OTC tolerance significantly differed between bacterial communities exposed to the three OTC concentrations, compared to communities grown in the absence of OTC, due to antibiotic-induced selective pressure.

PW262 Metagenomic survey and heavy metals' specific genes analysis of Surpentinite area in Taiwan

Bing-Mu Hsu[1]

[1]*National Chung Cheng University, Earth and environmental sciences, Minxung, Taiwan*

Background: The relatively high concentrations of more presenting heavy metal iron like lead, nickel, cobalt, and chromium were found in the serpentinite area in eastern Taiwan. It is a useful strategy to deal with the pollution of environmental heavy metal by studying the investigation of the interactions between microbial communities and environmental chemicals intensively.

Objectives: To understand the characterization of microbial communities and specific genes correlated with metabolisms of heavy metals in serpentinite area in Taiwan.

Methods: The serpentinite rock and soil samples were collected in Huatung Longitudinal Valley for investigating the microbial communities and quantitative evaluation of related functional genes. Bacterial 16S rRNA of V3-V4 gene region of the total samples were amplified and identified by next-generation sequencing.

Results: The dominant Phyla of samples from serpentinite samples are Proteobacteria, Actinobacteria, Bacteroidetes, Acidobacteria, and Firmicutes, while the special heavy metal-tolerant bacteria genera are included *Altererythrobacter*, *Nocardioides*, *Deinococcus*, and *Arthrobacter*. Based on the study of the bacterial community, the heavy metal resistance potential of each sample could be evaluated by the KEGG database. The outcomes indicated that the heavy metal gene expression of chromium, nickel, and cobalt from rock samples are higher comparative to soil samples in the serpentinite area.

PW263 The secret life and times of AmpC beta-lactamase antibiotic resistance genes in aquatic environments

Roelof Coertze[1], Carlos Bezuidenhout[1]

[1]*North-West University, Potchefstroom Campus, Microbiology, Potchefstroom, South Africa*

Background: AmpC beta-lactamase genes are common antibiotic resistance genes that require special attention once they have become mobilised on plasmids. Various studies have documented the detection of these genes in clinical settings. However, limited knowledge exists regarding both plasmid and genomic AmpC genes in aquatic environments.

Objectives: It was thus deemed important to review the extent of the gap in the literature regarding the prevalence of AmpC beta-lactamase genes in aquatic systems.

Methods: In this quest, a total of 27 databases were searched for peer-reviewed journal articles in this field. No date or language restrictions impacted this search but the focus was on the detection of AmpC beta-lactamase genes in environmental aquatic systems, including wastewater treatment plants.

Results: The result was 950 literature sources of which 50 were appropriate for full text analysis based on predetermined criteria. Studies on AmpC genes detection were traced in 23 countries. Of these studies, twenty-four focused on surface water, seventeen on wastewater, fourteen on sea water, and five on both surface and wastewater. The detection of AmpC genes was not generally the focus, as most studies aimed to detect antibiotic resistance genes in general. No surveillance, standardised detection methods and environmental limits currently exist for these genes; thus, due this gap, it is unlikely that such systems will be operationalized in the near future. However, the implications and dynamics of AmpC genes in aquatic systems remain unclear and require rigorous research to ensure the sustainability of environmental systems and human health.

PW264 Unraveling the composition of mineral water microbiota

Cristina García-Aljaro[1], Laia Caudet[1], Anicet R. Blanch[1], Francisco Lucena[1], Laura Sala-Comorera[1], Daniel Toribio[1], Belen Galofré[2], Gemma Saucedo[2], Carlos Vilaró[2], Sonia Fernández[3], David Baquero[3], Rosa Aznar[4], Maria A. Ruvira[4], Lidia Rodrigo-Torres[4], M.Carmen Macian[4], David R. Arahal[4], María J. Pujalte[4]

[1]*University of Barcelona, Genetics, Microbiology and Statistics, Barcelona, Spain*
[2]*Aigües de Barcelona, EMGCIA,S.A, Laboratori, Barcelona, Spain*
[3]*Cetaqua, Centro Tecnológico del Agua., Cornellà del Llobregat, Spain*
[4]*Colección Española de Cultivos Tipo (CECT), Universitat de València. , Departamento de Microbiología y Ecología, Valencia, Spain*

Background: Mineral waters are complex and highly unexplored environments with a high microbial diversity, with bacteria in different metabolic states.

Objectives: The aim of this work was to characterize mineral water bacterial communities using two different approaches that target bacteria in different physiological states: a) MALDI-TOF mass spectrometry (MS), to characterize the culturable communities and b) Illumina amplicon sequencing for the non-culturable.

Methods: Different mineral water brands were analysed 1 month after bottling. Culturable heterotrophic bacteria were isolated in WPCA (ISO) at 22 ± 2 °C for 72 h and identified using MALDI-TOF MS. The Bruker Daltonics Library and a customized library (Drinking Water Library, DWL) generated in our lab were used as reference database. For the non-culturable fraction, DNA was extracted from 6 l of water and Illumina 16S rRNA sequencing of the v4 region performed. DADA2 pipeline and SILVA database were used for analysis of the reads.

Results: The culturable heterotrophic communities were highly diverse and characteristic of each mineral water brand. Bruker Daltonics library allowed only identification of 30% of the isolates. The remaining were identified with the DWL. Among the culturable bacteria, the phylum Proteobacteria was the most abundant (around 85% of the isolates), followed by Actinobacteria Firmicutes and Bacteroidetes. Proteobacteria was also the most abundant phylum detected with Illumina sequencing (>99% of the reads). Important differences were observed at the different taxonomic levels especially at genus level between both approaches. Therefore, both approaches are complimentary for the characterization of the microbiota of mineral water environments.

PW265 Analysis of the oxygen barrier generated by a cathodic biofilm in a single chamber microbial fuel cell

Eloi Martinez Rabert[1], rebeca gonzalez-cabaleiro[1], albert guisasola[2], juan a. baeza[3]

[1]*University of Glasgow, Infrastructure and Environment, Glasgow, United Kingdom*
[2]*Autonomous University of Barcelona, Chemical, Biological and Environmental Engineering, Bellaterra, Spain*
[3]*Autonomous University of Barcelona, Chemical Engineering, Bellaterra, Spain*

Background: Single chamber microbial fuel cells (SC-MFC) can generate electricity concomitantly to biological oxidation of organic matter. In these systems, the solid anode acts as electron acceptor for the organic matter oxidation. The electrons flow from the anode to the cathode where the chemical reduction of oxygen takes place. If the diffusion of oxygen from the cathode reaches the anode, this reduces the efficiency of the overall system. Attachment of heterotrophic aerobic bacteria on the cathode surface is therefore necessary: it prevents the oxygen intrusion to the anode.

Objectives: The aim of this work is to study the oxygen barrier effect and stratification mechanism of the cathodic biofilm in a SC-MFC.

Methods: We have used a mathematical model of SC-MFC air-cathode to simulate the oxygen diffusion through the cathode layers. A model at the microscale level has been incorporated to analyse the heterogeneity of the cathodic biofilm.

Results: Simulation results predict that a thin biofilm on the cathode (≈ 4 μm) is enough to keep the oxygen concentration below 0.15 mg L^{-1} on the anode. With a thicker biofilm (≈ 20 μm) the maximum oxygen concentration on the anode is only 0.04 mg L^{-1}. Besides, possible endogenous activity of the population also helps the oxygen barrier effect. If the carbon source gets depleted, the oxygen barrier will persist for 30 more hours. The analysis of the biofilm structure revels competition between anaerobic and aerobic bacteria for the carbon source when oxygen gets depleted. This could reduce the oxygen barrier effect.

PW266 Some elements on detoxification pathway in Pseudomonas fluorescens under nitrosative stress

SEGOLENE DEPAYRAS[1], Thibault Chautrand[1], Tatiana Kondakova[2], Djouhar Souak[1], Rachel Duchesne[1], Mathilde Bouteiller[1], Julie Hardouin[3], Yoan Konto-ghiorghi[1], Olivier Maillot[1], Annabelle Merieau[1], Marc Feuilloley[1], Nicole Orange[1], Cecile Duclairoir-Poc[1]

[1]LMSM, Évreux, France
[2]Cronan Lab, Department of Microbiology, Urbana, United States
[3]Laboratory « Polymères, Biopolymères, Surfaces » , UMR 6270 CNRS, Rouen, France

Background: Nitrogen oxides (NO_x) are increasingly worrying atmospheric pollutants produced by anthropogenic activities. NO_x are closely linked to Reactive Oxygen Species (ROS) whose cellular targets and mechanisms of action are shared. The high reactivity of NO_x makes them toxic for humans and bacteria alike at high concentration, but nitric oxide (NO) is also produced by macrophages as an antibacterial agent. Thus, bacteria encounter these deleterious molecules through atmospheric pollution and during immune response. Some bacteria have acquired resistance mechanisms to nitrosative stress. *Pseudomonas fluorescens* is an ubiquitous bacterium, notably found in the atmosphere as well as in human, as pulmonary microbiota, where it is acting as a potential pathogen. These characteristics make it an interesting experimental model in the context of atmospheric pollution.

Objectives: The objective of this work is to determine the mechanisms used by bacteria to detect and respond to nitrosative stress. The flavohemoprotein Hmp seems to play a role in tolerance to nitrosative stress and was studied more thoroughly.

Methods: The *P. fluorescens* strain MFAF76a as well as a *Δhmp* mutant were exposed to 45ppm of gaseous NO_2 to simulate environmental pollution. Then, proteomic and transcriptomic profiles of these strains were done, as well as several physiological tests such as survivability and membrane permeabilization.

Results: Here, we report the identification of Hmp transcriptional regulator (HmpR) as a potential NO_x sensor in *P. fluorescens*. These results have also highlighted the importance of Hmp in the bacterial tolerance to NO_x.

PW267 FlashWeave: rapid inference of large-scale ecological networks from heterogeneous microbial sequencing data

Janko Tackmann[1], João Frederico Matias Rodrigues[1], Christian von Mering[1]

[1]*University Zürich, Institute of Molecular Life Sciences, Zürich, Switzerland*

Background: The recent explosion of metagenomic sequencing data necessitates tools for rapid computational analysis. While such software is becoming increasingly available for OTU mapping and clustering, co-occurrence-based prediction of microbial interactions is lagging behind.

Objectives: Simple correlation-based tools scaling to large data sets exist, yet these do not distinguish between direct and indirect interactions, resulting in excessive numbers of false positives. Approaches with better resolution, however, are so far highly limited in the size of data sets they can process. Furthermore, environmental and technical factors, though important confounders for microbial association signals, are rarely considered.

Methods: We present FlashWeave, a new software tool that adopts a flexible machine learning framework based on Probabilistic Graphical Models to infer highly resolved microbial interactions from heterogeneous, large-scale microbial abundance data sets with seamless integration of environmental and technical factors.

Results: FlashWeave is highly optimized for speed, scaling to tens of thousands OTUs and samples, outperforming six state-of-the art methods by up to three orders of magnitude. It further surpasses current methods in terms of accuracy on a wide collection of synthetic benchmark data sets. We apply FlashWeave to a cross-study data set of 69 818 publicly available human gut samples, resulting in one of the largest and most diverse models of microbial interactions in the human gut to date. The network reveals microbial hubs only characterized at the family level, constituting potential candidates for novel keystone taxa in the human gut, and shows indications of pronounced kin selection.

PW268 Effects of carbon source and C/N ratio on biodegradation of nitrous oxide

Kyung-Suk Cho[1], Hyung-Joo Park[1], Ji Hyeon Kwon[1], Yun-Yeong Lee[1], Jeonghee Yun[1]

[1]Ewha Woman's University, Environmental Science and Engineering, Seoul, Korea, Rep. of South

Background: Nitrous oxide (N_2O) is one of greenhouse gases with a global warming potential 310 times higher than that of carbon dioxide CO_2. N_2O participates in the stratospheric ozone layer depletion. The microbial reduction of N_2O to N_2 is only one known sink for N_2O in the biosphere. There is little information on N_2O biodegradation mechanisms, which limits the development of biotechnology for N_2O abatement.

Objectives: The effects of carbon source and C/N ratio on N_2O biodegradation was characterized.

Methods: The bacterial N_2O-degrading consortium was obtained by enrichment culture using activated sludge as an inoculum, and then the N_2O biodegradation by the consortium was characterized. The bacterial community structure was analyzed using high throughput sequencing based on 16S-rDNA.

Results: The N_2O-degrading consortium had functional genes for the reduction of NO_3^- (*narG*), NO_2^- (*nirK*) and NO (*norB*) as well as N_2O (*nosZ*). Its N_2O-reducing activity wasn't inhibited by high strength of N_2O (3,000 ppm). The consortium showed the highest N_2O-reducing activity when ethanol or sodium acetate was supplied as a carbon source. The N_2O-reduction rate by the consortium was decreased with increasing C/N ratio, and the optimum C/N ratio for N_2O-reduction was 80-130. The dominant bacteria in the consortium were *Tolumonas* (34.1%), *Azonexus* (11.6%), *Zoogloea* (9.9%), *Devosia* (7.5%), *Rhizobium* (4.6%), and *Pseudomonas* (2.5%). These results indicate that the N_2O-reducing consortium is a promising biocatalyst for the N_2O abatement biotechnology. The information about the effect of operation parameters on N_2O degradation can be used for the design of N_2O abatement bioprocess.

PW269 A study of ESBL genes in association and localization with mobile genetic elements among bacteria from natural aquatic environment

Insha Sultan[1], qazi mohd rizwanul haq[1]

[1]*jamia millia islamia, biosciences, NEW DELHI, India*

Background: Antibiotic resistance, widely recognized as a global issue, has increased the morbidity and mortality caused by bacterial infections, as well as the cost of treating infectious diseases. Resistance is a phenomenon originating from the retort of bacteria to the prevalent use of antibiotics and their occurrence in the environment. ESBLs consist of 3 major genetic groups: TEM, SHV, and CTX-M. The spread of ESBL genes through horizontal transfer of genes is associated with various mobile genetic elements, such as transposons, insertion sequences, and integrons which help there easy dissemination in the environment.

Objectives: The present study was carried out to determine the prevalence of ESBL genes along with mobile genetic elements among bacteria from pristine lakes of Jammu & Kashmir; India. It highlights the importance of understanding molecular biology of different mobile genetic elements in order to control the spread of antibiotic resistance.

Methods: Out of 201 isolates collected from 13 different sites, 38 were phenotypically positive for ESBL production according to Clinical and Laboratory Standard Institute (CLSI 2015). Furthermore, we studied association of ESBL genes with mobile elements as well as their localization in bacteria. Plasmid based replicon typing was also done.

Results: Molecular characterization of ESBL genes confirmed *bla*CTX-M and *bla*TEM genes in 25, 11 isolates respectively. The results of PCR amplification and sequencing confirmed the association of ESBL genes with genetic elements ISecp1, Tn3, Tn21, Int I and its gene cassette (Sul, qnrS). Molecular analysis of transconjugants showed successful transfer of mobile genetic elements.

PW270 Role of arbuscular mycorrhizal fungi associated to Ornithopus compressus on manganese toxicity and oxidative stress in shoots of wheat grown in an acidic cambisol of the Montado system, Portugal

Jorge M. S. Faria[1], Dora Teixeira[2;3], Ana Paula Pinto[1;3], Luís Alho[1;3], Isabel Brito[1;3], Mário Carvalho[1;3]

[1]ICAAM – Instituto de Ciências Agrárias e Ambientais Mediterrânicas, Évora University, Núcleo da Mitra, Ap. 94, 7002-554 Évora, Portugal
[2]HERCULES Laboratory, Évora University, Largo Marquês de Marialva 8, 7000-809 Évora, Portugal
[3]Science and Technology School of Évora University, Rua Romão Ramalho nº59, 7000-671 Évora, Portugal

Background: Increase of soil bioavailable manganese (Mn) due to soil acidity and winter weather conditions (waterlogging) is frequent in some cambisols of the portuguese Montado system, leading to heavy constraints on crop production. In untilled soils, where intact extraradical mycelium (ERM) grants an earlier and faster colonisation, the arbuscular mycorrhizal fungi (AMF) associated to an indigenous plant (*Ornithopus compressus*, ORN), can protect some crops against Mn toxicity, substantialy improving their growth[1,2].

Objectives: The protective effect of AMF colinization initiated from ERM associated to *O. compressus* was analysed on wheat growth, Mn and phosphorous (P) composition and on antioxidant enzymes activity.

Methods: Shoots of 3-week-old wheat grown after ORN under disturbed (disrupted ERM) or undisturbed (intact ERM) soils were compared to evaluate differences in (a) shoot growth, (b) Mn and P concentrations, quantified through ICP-MS, and (c) activity of ascorbate peroxidase, catalase, glutathione reductase, guaiacol peroxidase and superoxide dismutase[3].

Results: In undisturbed soil, wheat shoot growth increased more than 2 fold, shoot P contents more than doubled, Mn decreased below toxic concentrations and antioxidant enzyme activities decreased, indicating an AMF induced reduction of oxidative stress. The maintenaince of intact indigenous AMF mycelium network, by avoiding soil tillage, may be a very sucessfull and environmental friendly agricultural practice to avoid Mn toxicity in crop production at the portuguese Montado system.

1. Brito I, et al. (2014) Soil Biol Biochem, 68:78-84
2. Brito I, et al. (2018) Fungal Ecol, *in press*
3. Faria J, et al. (2018) Free Radical Bio Med, 120:S151

PW271 Metagenomics and metabolic profile of extant microbial community during remediation of heavily oil-polluted site

Chinedu Obieze[1], Chioma Blaise Chikere[2], Rasheed Adeleke[3], onyewhuchi akaranta[1;4]

[1]*University of Port Harcourt, Africa Centre of Excellence in Oilfield Chemicals Research, Port Harcourt, Nigeria*
[2]*University of Port Harcourt, Department of Microbiology, Port Harcourt, Nigeria*
[3]*Agricultural Research Council - Institute for Soil, Climate and Water, Microbiology, Pretoria, South Africa*
[4]*University of Port Harcourt, Department of Pure and Industrial Chemistry, Port Harcourt, Nigeria*

Background: Refining of crude oil under very primitive conditions referred to as artisanal refining has led to massive pollution of agricultural land, creeks and wetlands. Microbial response and their metabolic signatures to the presence of this pollutant in the environment have not been reported.

Objectives: The objectives of this study was to determine the microbial diversity, dynamics and metabolic signatures at different soil depths and as remediation of the polluted site progressed.

Methods: Microbial diversity was determined using Illumina Miseq while the metabolic profile was determined using gas chromatography-mass spectrometry. Other parameters analyzed included the total petroleum hydrocarbons, polycyclic aromatic hydrocarbons and the soil pH amongst others.

Results: Proteobacteria dominated at all depths of the polluted soils while the un-polluted soil was populated by Firmicutes. Prior to remediation, Proteobacteria, Acidobacteria and Chloroflexi were the most abundant phyla, but as remediation commenced dominance shifted to Proteobacteria alone with *Acidocella* being the most abundant genus. Principal component analysis showed a clustering of samples based on sampling time and depth. Bacterial biomarkers during early remediation were *Acidocella*, *Parvibaculum* and *Sulfuritalea* while at the 1m, 1.5m and 0-15cm depth (before remediation) were *Smithella*, *Pelolinea* and *Acidibacter*. Biomarker metabolites when remediation commenced included Hexadecane, Benzoic acid and Nonadecane. The study reveals that acidophilic organisms play important roles in the recovery of soils polluted by artisanal oil refining. Over 90% of the metabolites could not be mapped to KEGG pathways and will require further investigation to determine if they are potential dead-end products of degradation.

PW272 Diversity of arsenic-resistance genes from aquatic environment of Atacama Desert, Chile

Cristian Valenzuela[1], Ruben Moraga[2], Victor Campos[3]

[1]*University of Concepción, Faculty of Biological Science, Department of Microbiology, Concepción, Chile*
[2]*University Arturo Prat, Faculty of Renewable Natural Resources, Iquique, Chile*
[1]*University of Concepción, Faculty of Biological Science, Department of Microbiology, Concepción, Chile*

Background: The aquatic environments of the Atacama Desert (Chile), to characterized for a high concentration of mineral, due to the geological characteristics of sector. The most of these aquatic environments present, naturally, high concentrations of arsenic. Due its toxicity, to microorganisms present in these environments require mechanisms that allow them to growth in the presence of the metalloid.

Objectives: To determine the abundance and distribution of genes related to arsenic, in different aquatic environments of the Atacama Desert, as well as the diversity of prokaryotes.

Methods: Sediment samples were taken from salt flats Huasco, Ascotan, Atacama and Loa river. DNA obtained from sediments was used for high-throughput sequencing. The sequences were processed with bioinformatics tool to eliminate low quality sequences, and for assembly and annotation the sequences, as well as to obtain taxonomic profiles and presence of genes related to arsenic.

Results: The results show the presence of arsenic resistance genes (*ars*ABCM genes) in all sites. The *ars*B gene was the most abundant (40% -60%), except in el Huasco salt flat where *ars*A was the most abundant (42%). For the arsenic metabolic genes, the most abundant was the *aioA* gene (7.7% -28%), present in all the samples. The *arrA* gene was more abundant in Ascotan and Loa, in Atacama after the *aio*A gene the most abundant was the *arx*A gene. The most abundant phyla were the *Proteobacteria* and *Bacteroidetes*. The *Euryarchaeota* phylum was particularly abundant in the Loa.

PW273 Toxicity removal of arsenic compounds by a sequential aerobic biological treatment system

Javiera Ravanal[1], Carla Geraldine León Lemus[2], Luis Perira[1], Cristian Valenzuela[1], Victor Hernandez[1], Maria-Angelica Mondaca[2], Carlos Smith[1], Victor Campos[1]

[1]University of Concepción, Faculty of Biological Science, Department of Microbiology, Concepción , Chile
[2]University of Concepción, Faculty of Biological Science, Department of Microbiology, Concepción, Chile

Background: Organoarsenic compounds (OAC) have been widely used as food additive in poultry industry and as a pesticide. Use of poultry manure like fertilizer and use of arsenic pesticides, incorporating considerable amounts of arsenic (As) to farm fields and groundwater. Ingestion of As contaminated water above this level cause cancer and other health problems.

Objectives: To implement a sequential aerobic biological treatment system (SABTS), to reduce the toxicity of organoarsenic compounds from an artificial effluent (AE).

Methods: SABTS was implemented in two stages at laboratory scale. First stage (S1) was a batch bioreactor where a bacterial consortium was cultivated in order to reduce the OAC from an AE (supplemented with roxarsone (0.5 mM), nitarsone (0.5 mM) and monosodium methanoarsonate (0.5 mM)). Second stage (S2) was a feed-batch bioreactor where *Pseudomonas arsenicoxydans* was cultivated in a medium adapted for Microbially-Induced-Calcite-Precipitation (MICP), in order to remove the arsenic present in the affluent (12%v/v of S1 effluent) by oxidation of arsenite and co-precipitation of arsenic-calcite. Growth kinetics was studied by OD (600 nm) and micro-drop technique. Arsenic concentration was determined by HPLC-HG-AAS. Toxicity of affluent was evaluated by means Human Umbilical Vein Endothelial Cells (HUVECs).

Results: Reduction of OAC was 96,70±0,042% at 144 h of S1 operation. Removal of arsenic was 100% (0.000±0.005mg·l^{-1}) after 72h of S2 operation with an arsenic removal rate of 0.033±8.750e-007 mg·l^{-1}·h^{-1}. HUVECs viability was 99.3±0.51% after exposition to the final effluent. SABTS with arsenite-oxidation/calcite-arsenic co-precipitation biotechnology was able to reduce the toxicity of arsenical compounds.

PW274 High pCO2 driven ocean acidification on open ocean bacterial communities during a microcosm experiment in the Eastern Tropical South Pacific (ETSP) off northern Chile

Paulina Aguayo Venegas[1], carlos Henríquez [2], francisca olivares[2], Rodrigo De la Iglesia[3], Osvaldo Ulloa[2], Cristian Vargas[1]

[1]Millennium Institute of Oceanography (IMO), Universidad de Concepción, Concepcion, Chile, Department of Aquatic System, Faculty of Environmental Sciences & Environmental Sciences Center EULA Chile, Universidad de Concepción, Concepción, Chile, concepción, Chile
[2]Millennium Institute of Oceanography (IMO), Universidad de Concepción, Concepcion, Chile, Department of Oceanography, Universidad de Concepción, Concepción, Chile, Chile
[3]Department of Molecular Genetics and Microbiology, Pontificia Universidad Católica de Chile. , Santiago, Chile, Chile

Background: Ocean acidification (OA) is one of the most important global environmental threats confronting marine life. Due to the increasing anthropogenic CO_2 emissions, OA is progressing rapidly around the world and causing significant impacts on biodiversity. However, the response of bacterial communities major players on the marine biogeochemical cycling and ecosystem functioning to OA is still not well understood

Objectives: To assess the effect of low pH/high pCO_2 conditions over the abundance and genetic diversity of naturally marine bacterial communities through a microcosm experiment carried out during a research expedition to the Eastern Tropical South Pacific (ETSP) off northern Chile

Methods: The composition and relative abundance of bacteria were analyzed by massive sequencing of the V4 region of the 16S rRNA small sub-unit encoding gene. The results were contrasted with the cellular abundances obtained by flow cytometry and physico-chemical variables associated

Results: The results were contrasted with cellular abundances obtained by flow cytometry and physico-chemical variables associated. SAR11 (Alphaproteobacteria),SAR324 (Deltaproteobacteria) and Artic96BD-19 (Gammaproteobacteria) were the most abundant taxa through the water column. Genetic analysis evidenced a predominance of the order *Alteromonadales, Rhodobacterales* in both control and experimental pCO_2/low pH conditions. Interestingly, at low pH/high pCO_2 levels the *Planctomycetales* were detected at the end of the experiment. These organisms are key players in the nitrogen cycle in low oxygen waters, but not in surface waters. In consequence, our findings suggest that changes OA could have the potential of causing changes in bacterial composition, and therefore on the nitrogen biogeochemical cycle.

PW275 Effect of bacteriophage on the co-cultivation of Alexandrium tamarense with symbiotic bacteria

Chang Soo Lee[1], Kiwoon Baek[2], Ahyoung Choi[3]

[1] Nakdonggang National Institute of Biological Resources, Culture Techniques Research Division, Sangju, Korea, Rep. of South
[2]Nakdonggang National Institute of Biological Resources, Bacterial Resources Research Division, Sangju, Korea, Rep. of South
[3]Nakdonggang National Institute of Biological Resources, Culture Techniques Research Division, Sangju, Korea, Rep. of South

Background: Harmful algal blooms (HABs) are important environmental issues to cause the contaminations of water resources and economic damages in fishery industries. *Alexandrium tamarense* is one of major compositions in the marine HABs to cause human illness clinically known as paralytic shellfish poisoning.

Objectives: In this study, we have focused on the microbial roles of symbiotic bacteria and bacteriophage in the growth of *Alexandrium tamarense*.

Methods: A bacteriophage, IMCC9565P, was isolated from symbiotic bacteria, *Planktotalea arctica*. Effects of symbiotic bacteria and its bacteriophage on the growth of *A. tamarense* were investigated by co-culturing with algae, bacteria, and bacteriophage using 48 well plate.

Results: When 1.0 x 10^3 cell/mL of *A. tamarense* was co-cultured with 1.0 x 10^5 cell/mL *P. arctica*, the cell-division of *A. tamarense* was promoted during 3 days after inoculation. The cell concentration of *A. tamarense* was increased to 1.0 x 10^4 cell/mL after 8 days, indicating that *P. arctica* positively contributed to algal growth. In order to determine effects of IMCC9565P bacteriophage was inoculated into the algal-bacterial co-culture. The plaque-forming lysis of bacterial host, *P. arctica*, was clearly detected on the bottom of 48 well plate during 2 days after inoculation. The inoculation of bacteriophage into the co-culture with *A. tamarense* and *P. arctica* resulted in the interruption of cell division and rapid cell deaths of *A. tamarense.* The results indicated that the lysis of symbiotic bacteria by bacteriophage can effect on the dynamics of harmful algal growth by dramatic disruptions of algal-bacterial consortia.

PW276 Biomonitoring of photobioreactors established to investigate the impact of toxic load and pollutants variation in coking wastewater treatment: Harmonization between microalgae and wastewater microbiome

Mariam Hassan[1], Tamer Essam[1], Salwa Megahed[1;2]

[1]Faculty of Pharmacy, Cairo University, Microbiology and Immunology, Egypt
[2]Faculty of Pharmacy, October University for Modern Sciences and Arts, Microbiology and Immunology

Background: The investigation of microbial community structures is a significant way to understand biodegradation capacities in biological wastewater treatment.

Objectives: The microbiome and the treatment efficiency of algal-bacterial systems were biomonitored using different bioassays (phytotoxicity, Artemia-toxicity, cytotoxicity, algal-bacterial ratio and settleability).

Methods: Photo-bioreactors A, B and C received real coking-wastewater as influent with COD 776±56, 1229±85 and 2033±27 mg/l, respectively. In phase-1 phenol was added to the influent, while dichlorophenol was added in combination with phenol in phase-2. COD removal%, phenol and dichlorophenol concentrations were monitored.

Results: All systems efficiently detoxified the influents in phase-1. In phase-2, Systems B and C failed to detoxify the influents. Illumina-sequencing generated 2119749 sequences of 16S-rRNA gene from 21 influent and effluent samples. The number of observed species was significantly lower in effluent than influent samples, some taxa dominated and contributed to the systems' performance. Significant difference in the microbial-diversity between influent and effluent was detected. *Proteobacteria* (78%), *Firmicutes* (12%), *Bacteroidetes* (5%) and *Deferribacteres* (2%) were the dominant phyla in influent samples. In effluent samples *Proteobacteria* (68%) and *Bacteroidetes* (25%) dominated. Failure in treatment process in systems B and C at phase-2 was accompanied with significant difference in the microbial-diversity. The co-culture of microalgae shifted the microbiota and promoted the activity of genera affiliated to *Chitinophagaceae*, *Pseudomonadaceae* and *Xanthomonadaceae* families. These bacteria are known for their catabolic-diversity and xenobiotic degradation. The superiority of algal-bacterial systems for coking-wastewater treatment was confirmed as co-culture of microalgae eradicated pathogenic bacteria (*Arcobacter* and *Legionella* genera) in efficiently treated effluents.

PW278 Phenotypic characterization of antimicrobial resistant enterobacteriaceae isolates recovered from vegetables, hospital effluents and river water

Lindelwa Mpaka[1;2], Anthony Ifeanyi Okoh[1;2], martins adefisoye[1;2]

[1]SA-MRC Microbial Water Quality Monitoring Centre, University of Fort Hare, Pivate Bag X1314, Alice 5700, Eastern Cape, South Africa, Biochemistry and Microbiology, Alice, South Africa
[2]Applied and Environmental Microbiology Research Group, University of Fort Hare, Alice, Eastern Cape, South Africa, Biochemistry and Microbiology, Alice, South Africa

Background: The emergence and spread of antimicrobial-resistant Enterobacteriaceae is considerably the third-largest threat to public health and food security worldwide and hence has become an important challenge in disease control. Globally, about 700 000 deaths every year can be accredited to antimicrobial resistance.

Objectives: The main aim of this study is to evaluate the incidence and antibiogram fingerprints of Enterobacteriaceae recovered from hospital effluents, river water and vegetables in the Eastern Cape Province.

Methods: Isolates were confirmed by MALDI-TOF MS analysis. Eighteen commercial antibiotics from ten different antimicrobial classes were used to determine antibiogram profiles of the MALDI-TOF MS confirmed isolates using disc diffusion technique.

Results: About 75.5% (25/33) of the presumptive *Enterobacter* spp. were confirmed by MALDI-TOF MS with 79.2% (19/24), 66.7% (2/3), 66.7% (4/6) from vegetables, hospital effluents and river water respectively. Likewise, about 77.8% (21/27) were confirmed as *Citrobacter* spp. of which 92.3% (12/13), 66.7% (2/3) and 63.6% (7/11) were from vegetables, hospital effluents and river water respectively. From the MALDI-TOF MS confirmed isolates, 85% (40/47), 92% (23/25), 100% (21/21) and 100% (12/12) of *E. coli*, *Enterobacter* spp., *Citrobacter* spp. and *Klebsiella* spp. isolates displayed resistance against ampicillin respectively. Our results confirm the incidence of antimicrobial resistant Enterobacteriaceae strains in the water-agroecosystem nexus of the Eastern Cape and South Africa in general. The high frequencies of colistin resistance among the isolates underscores the need to develop better management strategies and interventions to forestall the negative impact of their spread on human health.

PW279 Prokaryotic communities in air particulate matter from Cyprus

Jörn Wehking[1], Petya Yordanova[1], Beatriz Sánchez-Parra[1], Stefanie Maier[1], Daniel Pickersgill[1], Jean Sciare[2], Ulrich Pöschl[1], Bettina Weber[1], Janine Fröhlich-Nowoisky[1]

[1]Max-Planck Institute for Chemistry, Multiphase Chemistry, Mainz, Germany
[2]The Cyprus Institute, Atmosphere and Climate Division, Aglantzia, Nicosia, Cyprus

Background: Airborne prokaryotes and other primary biological aerosol particles are ubiquitous in the atmosphere influencing atmospheric chemistry and physics, the biosphere, climate, and health.

The sources of airborne prokaryotes and environmental factors that influence their diversity and dynamics are not well characterized.

Objectives: Investigate the diversity of bacteria and archaea in aerosol samples and two possible sources, microbial surface communities and soil.

Methods: Aerosol, microbial surface community and soil samples were collected during the INUIT 2016 campaign in Cyprus, in the Eastern Mediterranean. Total DNA was extracted and sequenced by shotgun metagenomics.

Results: We found differences in composition and abundances, of the prokaryotic communities between aerosol and source samples, suggesting that microbial surface communities and soil were not the primary sources of the airborne prokaryotes.

Moreover, we observed that the composition of airborne bacteria stayed preponderant constant over the campaign and was mainly comprised by Actinobacteria, Bacteroidetes, Firmicutes and Proteobacteria. Airborne archaea were less abundant and species-rich than bacteria. Archaea were dominated by Methanobacteria, suggesting anthropogenic influences. Additionally, we found that the composition of airborne prokaryotes was affected by rain and dust events. Overall, this study gains insight into the sources and environmental factors that influence the prokaryotic part of the atmospheric microbiome.

PW280 Do singals produced by rhizosphere-living bacteria affect the behavior of a bacterial biocontrol agent?

Ana Bejarano[1], Gerardo Puopolo[2], Ilaria Pertot[1;2]

[1]Center Agriculture, Food, Environment, University of Trento, San Michele all'Adige, Italy
[2]Research and Innovation Centre, Fondazione Edmund Mach, Department of Sustainable Ecosystems & Bioresources, San Michele all'Adige, Italy

Background: To make agriculture more sustainable**,** bacterial biocontrol agents (BBCAs) are promising alternatives to the use of chemicals for the control of plant pathogens. However, their efficacy is often inconsistent in field due to their low persistence in the environment. In this regard, getting deep knowledge on the interactions between BBCAs with resident bacteria may improve their persistence.

Objectives: To evaluate how chemical signals produced by Gram-negative bacteria modulate the inhibitory activity of a model BBCA, *Lysobacter capsici* AZ78 (AZ78).

Methods: AZ78 genome was mined to determine the presence of cell-cell signaling systems. The effect of chemical signals produced by Gram-negative bacteria such as N-acyl-homoserine lactones (AHLs), diffusible soluble factors (DSFs) and diffusible factors (DF) on AZ78 inhibitory activity was determined *in vitro* including the signals in the growth medium used in classic dual-culture assays.

Results: An incomplete AHL quorum-sensing system and two complete cell-cell signaling systems based on DSFs and DFs were identified in AZ78 genome. Noteworthy, a gene encoding a putative DSF synthase was found for the first time in a member of *L. capsici* species. Exogenous addition of AHLs, DSF and DF in the growth medium, modulated the AZ78 inhibitory activity. Specifically, AHLs or the 3-hydroxybenzoic acid (DF) enhanced similarly the AZ78 inhibitory activity. On the contrary, a decrease of AZ78 inhibitory activity was observed when 13-methyltetradecanoic acid (DSF) or 4-hydroxybenzoic acid (DF) were included in the growth medium. Further investigations at molecular levels will be performed to unravel molecular patterns modulated in AZ78.

PW281 Land use and its influence on soil bacterial diversity

Abisola Sholeye[1], Bernard Ojuederie[2;2;2;2]

[1]*North-West University, Microbiology, MAFIKENG, South Africa*
[2]*North-West University, Mafikeng Cmpus*

Background: Reduction in the organic matter content of the soil has been recognised to influence soil fertility hence fertilizer application by farmers to boost crop production. This is one of the major factors affecting microbial abundance in the soil. The soil microorganisms perform important functions in maintaining the balance in the ecosystem, as well as the fertility of the soil.

Objectives: Thus, in this study, the effect of land use on soil microbial diversity in two agricultural sites and one native indigenous forest in South Africa was determined. It is hypothesized that the forest land would be more biologically diverse with microorganisms due to reduced anthropogenic activities

Methods: The metagenomics methos was used to analyse soil samples

Results: The results showed that the agricultural lands were abundant in the classes *Proteobacteria*, *Actinobacteria*, *Bacteroidetes* and *Acidobacteria* with relative abundance values of 47.3%, 17%, 8.2% and 7.1% respectively while the indigenous native forests were abundant in the classes *Proteobacteria*, *Verrucomicrobia*, *Acidobacteria* and *Actinobacteria* with relative abundance values of 35.5%, 27.8 %, 23.7% and 5.8% respectively. The forest soils also had a higher microbial species richness than the agricultural soils. However, Limpopo agricultural soil had a higher diversity index than the forest site with an average Shannon Diversity index of 6.5825 as against the forest average Shannon diversity index of 6.429)

PW282 The role of soil bacteria at high temperatures

Margarida Santana[1;1], Maria C Portillo[2], Rui Ferreira[3], Cristina Cruz[1], Juan M Gonzalez[4]

[1]*ce3c, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal*
[2]*Universitat Rovira I Virgili Tarragona, Tarragona, Spain*
[3]*Centro de Química da Madeira, Universidade da Madeira, Portugal*
[4]*Instituto de Recursos Naturales Y Agrobíologia, Sevilla, Spain*

Background: Soils with poor plant cover are exposed to intense solar radiation which leads to increasing temperatures at top soils, well above the optimum for commonly studied mesophilic soil bacteria. At South Europe, values above 40ºC are frequently observed in top-soils and those values are expected to increase, since climate changes are incrementing high temperature events. Microbial activity at upper soil layers has been suggested to be highly reduced during such extreme temperature events. However, recent reports highlighted the occurrence of peaks of enzymatic activity in soils at high temperature (55ºC -75ºC) and the ubiquitous presence of thermophilic bacteria (STB).

Objectives: To assess the role of STB, which are mostly present in temperate soils as viable cells, as potential participants of soil biogeochemical reactions.

Methods: Composite soil samples were incubated in Nutrient Broth at different temperatures with or without copper. Sulfate and ammonium produced were determined by standard procedures. In situ protease and glucosidase activities in natural soil samples were determined using fluorogenic substrates.

Results: Thermophilic bacteria of the Firmicutes phylum, represented mainly by *Geobacillus* species were able to release significant quantities of sulfate and ammonium under high temperature conditions as a result of a dissimilatory organic-sulfur mineralization process, suggesting that STB can be actively involved in C, N and S cycling in soil upper layers and refuting the prevailing view of bacteria as poor S-mineralizers. Usual phytosanitary treatments in agricultural lands influence STB communities and sulfate release in soils with consequences to natural soil fertilization.

PW283 High-resolution mapping of the Israeli plant microbiome: A collaboration between the educational and scientific community

Maor Knafo[1], Shahar Rezenman[1]

[1]*Weizmann Institute of Science, biomolecular sciences , Rehovot, Israel*

Background: The plant microbiome plays a significant role in a myriad of ecosystems and holds great potential for biotechnology, discovery of naturally-occurring compounds, and exploration of species. However, our knowledge of its diversity and composition is restricted due to under-sampling.

Objectives: We have embarked on a nationwide project to map the Israeli plant-microbiota and create a novel plant and soil microbiome database. The project is led by scientists in collaboration with high schools across the country, a partnership that allows us to obtain a large number of samples from varied ecosystems while exposing students and teachers to cutting edge research.

Methods: We are currently analyzing data from a small-scale pilot in which leaves, flowers, roots and soil were sampled by students in the field. The collected samples have undergone a stepwise examination that consists of 16S and ITS community analysis using numerous pipelines employing state of the art techniques. In order to obtain the most accurate representation of the microbial community in the pilot samples, we are also performing 3rd generation sequencing of full length 16S/ITS transcripts.

Results: By comparing the results derived from multiple pipelines to the aforementioned ground truth community structure, we can determine which pipeline is optimal for use in future analyses.

We believe that the scope of this project coupled with its innovative sampling and analysis methods will be valuable for future studies of the plant and soil microbiome while also supporting high-level scientific education.

PW284 Providencia stuartii socialization confers resistance to environmental cues but can be challenged by bio-inspired peptides

Julie Lopes[1], Tetreau Guillaume[1], Bruyat Pierrick[2], El Khatib Mariam[1], Pounot Kévin[1;3], Vivaudou Michel[1], Renard Pierre-Yves[2], Jean Ludovic[2], Colletier Jacques-Philippe[1]

[1]*Institut de Biologie Structurale, Grenoble, France*
[2]*Laboratoire COBRA UMR CNRS 6014, Université de Rouen, Mont-Saint-Aignan, France*
[3]*Institut Max von Laue - Paul Langevin, Grenoble, France*

Background: *Providencia stuartii* is a Gram-negative pathogen involved in urinary tract infections. It is characterized by a stringent antibiotic-resistance phenotype and by a highly social behaviour, forming floating communities of cells (FCC) during the exponential phase that later sediment to form surface-attached biofilms (SAB). It was shown that *P. stuartii* porins play an active role in the formation of FCC, enabling direct cell-to-cell contact through self-matching interactions that result in the formation of dimers of porins trimers (or DOTs) between adjacent cells.

Objectives: In pathophysiological conditions, *P. stuartii* is exposed to high concentrations of urea and ammonia, and subjected to large pH variations. Hence, our first objective was to characterize *in vivo* the effect of these environmental cues on the formation of FCC and SAB. We attempted to challenge bacterial socialization before formation of SAB by inhibiting DOT formation.

Methods: *P. stuartii* cells were subjected to various environmental cues, and FCC and SAB formation were monitored by optical density measurements and epifluorescence microscopy. In parallel, we synthetized peptides mimicking the DOT interface and tested their effect on *P. stuartii* growth and the displayed phenotypes.

Results: Formation of FCC and SAB protects *P. stuartii cells* from harmful urinary tract metabolites such as urea and ammonia and enable growth within a large pH range. Peptides mimicking DOT interfaces are not able to disrupt FCC but their grafting with a coumarin moiety appears as a promising strategy to reveal – and possibly diagnose – *P. stuartii* infections.

PW285 Facets of diazotrophy in the OMZ off Peru revisited: what we could not see from a single marker gene approach

Christian Christiansen[1], Carolin Löscher[1]

[1]*Syddansk Universitet, Nordcee, Odense, Denmark*

Background: Biological dinitrogen (N2) fixation is the pathway making the large pool of atmospheric N2 available to marine life. Besides direct rate measurements, a common approach to explore the potential for N2 fixation in the ocean is a mining based on molecular genetic methods targeting the key functional gene nifH, coding for a subunit of the nitrogenase reductase. As novel sequencing and single cell techniques improved, our knowledge on the diversity of marine N2 fixers grew exponentially.

Objectives: One aspect of N2 fixation in the ocean is commonly left aside. This is the existence of two alternative types of nitrogenases, which are beside the Nif, the Anf and the Vnf, which differ in metal co-enzymes, as well as regarding their operon structure and composition. Besides looking at Nif, we explored Vnf and Anf to determine the ecological and evolutionary history of those.

Methods: We screened a set of six metagenomes and -transcriptomes from a sulfidic water patch from the oxygen minimum zone off Peru for genes involved in N2 fixation. Genes related to all three nitrogenases, and generally increased diversity as compared to our previous nifH- based study from the same waters.

Results: While we could not confirm gene expression of the alternative nitrogenases from our transcriptomes, we detected additional diazotrophs involved in N2 fixation. We suggest that alternative nitrogenases may not be used under conditions present in those waters, however, depending on trace metal limitation in the future they may become active.

PW286 Role of copper in bacterial predation

Francisco Javier Contreras-Moreno[1], Juana Pérez[1], Gonzalo Marinez-Navajas[1], Natalia I. Garcia-Tomsig[1], Aurelio Moraleda-Munoz[1], Jose Munoz-Dorado[1]

[1]*Faculty of Sciences of the UGR, Granada, Spain*

Background: *Myxococcus xanthus* is a soil bacterium with a complex social behavior. The communities of cells are able to cooperatively prey on other microorganisms. They produce a large amount and variety of secondary metabolites and hydrolytic enzymes to kill the prey, degrade their macromolecules, and feed on the released components to grow. On the other hand, a large number of genes involved in copper detoxification have been found in the *M. xanthus* genome, although this bacterium is not especially resistant to copper. Taking into consideration that eukaryotic predators and macrophages use copper to kill bacteria that they have engulfed, we have hypothesized that some copper detoxification systems of *M. xanthus* could somehow be involved in predation.

Objectives: For this reason, we have studied whether copper is involved in predation of *M. xanthus* on *Sinorhizobium meliloti*.

Methods: To investigate the role of copper on predation we have used genetic and microbiological methodologies, such as construction of *M. xanthus* strains harboring fusions between genes involved in copper detoxification and *lacZ*, deletion mutants, observations with light and electron microscopes, and quantification of cell viability after co-culture of predator and prey.

Results: The results obtained have revealed that copper accumulates in the interface prey-predator. Thus, *M. xanthus* genes whose expression depend exclusively on copper are expressed at higher levels in this region. Similarly, melanin produced by *S. meliloti* is also accumulated at the interface. Data about viability of the prey in the presence of copper will be presented at the meeting.

PW287 The Myxococcus xanthus predatosome and Sinorhizobium meliloti defensome

Aurelio Moraleda-Munoz[1], Francisco Javier Contreras-Moreno[1], Jared Schrader[2], Penelope Higgs[2], Jose Munoz-Dorado[1], Juana Pérez[1]

[1]*Faculty of Sciences, Department of Microbiology, Spain*
[2]*College of Liberal Arts and Sciences, Biological Building, Detroit (MI), United States*

Background: *Myxococcus xanthus* has a multicellular lifestyle that includes social predation against a variety of prey. During predation it uses diverse strategies depending on certain characteristics of the prey and induces different defensive responses (Pérez et al., 2011; Pérez et al., 2014).

Pérez et al. (2011). *Microb Biotechnol.* 4: 175-183.
Pérez et al. (2014). *Env. Microbiol.* 16: 2341-2350.

Objectives:

1. Elucidation of *M. xanthus* predatosome: genes involved in the attack, lysis, and killing.
2. Study of *S. meliloti* defensome: genes induced during the predatory process.

Methods: *S. meliloti* Rm1021 (non-mucoid) and 1021R (mucoid) were grown in solid medium and incubated for 24 hours. After this time drops of *M. xanthus* DK1622 were deposited over the *S. meliloti* cultures. The cell mixtures and controls were collected at different times. RNA and cDNA were obtained for RNA-seq massive sequencing by using NextSeq 500 Illumina. Differential expression analyses were carried out by using different bioinformatic programs.

Results: The *M. xanthus* predatosome versus *S. meliloti* Rm1021 is induced mainly at 2 hours where the overexpressed genes can be grouped in five categories: secondary metabolites production, lytic enzymes, outer membrane and outer vesicles associated proteins, transcriptional regulators, and iron importer genes. The *S. meliloti* defensome at early times includes genes of the regulon of the iron responsive regulator *rirA*, whose products are involved in the synthesis and uptake of siderophores, or in the uptake of other iron sources. Studies with different mutants indicate that the competition for iron is an essential predator-prey interplay.

PW288 Evidence for the Evolution, Clonal Expansion and Global Dissemination of Water Treatment-Resistant Naturalized Strains of Escherichia coli in Wastewater

Shuai Zhi[1], Norman Neumann[1], Paul Stothard[2], Graham Banting[1], Nicholas Ashbolt[1], Simon Otto[1], Sylvia Checkley[3], Kelsey Mayer[3]

[1]*University of Alberta, School of Public Health, Edmonton, Canada*
[2]*University of Alberta, Faculty of Agricultural, Life and Environmental Sciences, Edmonton, Canada*
[3]*University of Alberta, Faculty of Veterinary Medicine, Department of Ecosystem and Public Health, Calgary, Canada*

Background: Recent research suggests that *E. coli* may represent a species complex, composed of genetically distinct enteric and cryptic clades. We previously demonstrated the existence of naturalized strains of *E. coli* in wastewater.

Objectives: Herein we perform an in-depth comparative whole genome analysis of these strains (n=17).

Methods: Phylogenomic, whole genome similarity, unique gene, and functional genomic analysis were performed on these wastewater *E. coli* strains.

Results: The result demonstrated that 14 of the Canadian *E. coli* strains, isolated from geographically separated wastewater treatment plants, were virtually identical at the core genome and were >96% similar at the whole genome level. Remarkably, these strains were shown to be extremely similar to an *E. coli* isolated from wastewater in Switzerland, suggesting a global distribution of these strains. The genomes of three other Canadian wastewater strains were very similar to the genomes of *E. coli* isolates collected from U.S. wastewater samples. Based on phylogenetic analysis, wastewater strains from Canada, the U.S. and Switzerland formed a clade separate from other phylogroups. All Canadian, Swiss and U.S. wastewater strains possessed a common SNP biomarker pattern across their genomes. The remarkable degree of similarity between all wastewater strains from Canada, Switzerland and the U.S. suggests the evolution and global-dissemination of water treatment-resistant clone of *E. coli*. These finding, along with others, raise some important concerns about the potential for emergence of *E. coli* pathotypes resistant to water-treatment - exposing a breach in the most fundamental public health intervention strategies for control of waterborne infectious disease.

PW289 Carbon remineralization in an Antarctic polynya revealed by genomic reconstruction and metatranscriptomic analyses

So-Jeong Kim[1], Jong-Geol Kim[2], Sang-Hoon Lee[3], Soo-Je Park[4], Joo-Han Gwak[2], Man-Young Jung[5], Won-Hyung Chung[6], Eun-Jin Yang[3], Jisoo Park[3], Jinyoung Jung[3], Yoonsoo Hahn[7], Jang-Cheon Cho[8], Eugene L. Madsen[9], Francisco Rodriguez-Valera[10], Jung-Ho Hyun[11], Seung-Yeon Choi[2], Lorraine A. Bellosillo[2], Sung-Keun Rhee[2]

[1]Korea Institute of Geoscience and Mineral Resources, Geologic Environment Research Division, Daejeon, Korea, Rep. of South
[2]Chungbuk National University, Department of Microbiology, Cheongju, Korea, Rep. of South
[3]Korea Polar Research Institute, Division of Polar Ocean Environment, Incheon, Korea, Rep. of South
[4]Jeju National University, Department of Biology, Jeju, Korea, Rep. of South
[5]University of Vienna, Department of Microbial Ecology, Vienna, Austria
[6]Korea Food Research Institute, Research Group of Gut Microbiome, Sungnam, Korea, Rep. of South
[7]Chung-Ang University, Department of Life Science, Seoul, Korea, Rep. of South
[8]Inha University, Department of Biological Sciences, Incheon, Korea, Rep. of South
[9]Cornell University, Department of Microbiology
[10]Universidad Miguel Hernandez, Campus de San Juan, Evolutionary Genomics Group, San Juan, Alicante, Spain
[11]Hanyang University ERICA Campus, Department of Marine Science and Convergence Engineering, Ansan, Korea, Rep. of South

Background: Southern Ocean Polynyas are regions of intense primary production, mainly by *Phaeocystis antarctica*. However, in the Amundsen Sea, most of this organic carbon does not reach the sediment but is degraded in the water column due to high bacterial heterotrophic activity.

Objectives: Physiological properties and biochemical pathways of key heterotrophs can be predicted using genomes reconstructed by *de novo* assembly and analysis of environmental mRNA sequence reads to evaluate *in situ* activities

Methods: We reconstructed 12 key bacterial genomes from different phases of bloom and analyzed expression of genes involved in organic carbon remineralization.

Results: *Polaribacter* belonging to *Bacteroidetes* was dominant in the peak phase, and its transcriptional activity was high(48.9% / total mRNA reads). Two dominant *Polaribacter* bins potentially utilize major polymers in *P. antarctica*, chrysolaminarin and xylan, with a distinct set of glycosyl hydrolases. In the decline phase, *Gammaproteobacteria*(Ant4D3, SUP05 and SAR92), with the potential to utilize low molecular weight-dissolved organic matter including compatible solutes, was increased. The versatility of SAR92 and Ant4D3 clades of *Gammaproteobacteria* may contribute to their abundance in organic carbon-rich polynya waters. This study revealed the unique metabolic potential of dominant bacteria of Antarctic polynya at a finer taxonomic level. The information can be used to predict temporal community succession linked to the availability of substrates derived from the *P. antarctica* bloom. Global warming has resulted in compositional changes in phytoplankton from *P. antarctica* to diatoms and, thus, parallel studies in various polynyas are required to predict global warming-related changes in carbon remineralization.

PW290 Dominant nitrogen-transformation pathway and key ammonia-oxidizing microbial species of simultaneous nitrification and denitrification in the microaerobic activated sludge process for sewage treatment

Shaokui Zheng[1]

[1]*Beijing Normal University, School of Environment, Beijing, China*

Background: The microaerobic activated sludge (MAS) process is characterized by simultaneous nitrification and denitrification reaction and bulky sludge flocs in a one-stage microaerobic reactor. We demonstrated a shorter aeration tank hydraulic retention time (3 h), higher biomass concentration (6.3 $g \cdot L^{-1}$), 40% reduction in aeration energy consumption, and 2-3 times higher volumetric loading rate with the MAS process than those obtained in the conventional anoxic/oxic (A/O) biological nitrogen removal process, when no significant difference in pollutant removal performance was observed during synthetic sewage treatment.

Objectives: With the conventional A/O system as the control of the MAS system at a normal loading level, this study investigated the effect of the aerobic and microaerobic DO levels in both systems on the dominant ammonia-oxidizing and dinitrogen (N_2)–producing mechanism, and the dominant ammonia-oxidizing bacteria (AOB) species. Furthermore, the effect of the normal and high loading levels in the MAS system were investigated.

Methods: The molecular techniques and comprehensive nitrogen-transformation activity test were used to investige the dominant nitrogen-transformation mechanisms in this study.

Results: The DO levels in both systems did not significantly affect the dominant ammonia-oxidizing mechanism (i.e., a combination of aerobic heterotrophic and chemolithotrophic pathways) and the dominant AOB species. Nor did the loading levels in the MAS system affect the dominant ammonia-oxidizing mechanism significantly, but they did impact the dominant AOB species. N2 is predominantly produced from microaerobic denitrification in the MAS system, when it is produced from anoxic denitrification in the conventional A/O system.

PW291 Isolation of two aerobic methane-oxidizing enrichment cultures from the Indian Ocean Hydrothermal System

Lorraine A. Bellosillo[1], Samuel I. Awala[1], Seung-Yeon Choi[1], Ngoc-Loi Nguyen[1], Woon-Jong Yu[1], Sung-Keun Rhee[1]

[1]Chungbuk National University, College of Natural Science, Microbiology Department, Cheongju, Korea, Rep. of South

Background: The deep-sea hydrothermal vent systems are considered a major source and sink of methane (CH_4), a potent greenhouse gas. Methane is frequently consumed through aerobic and anaerobic process.

Objectives: Since the deep-sea hydrothermal vent systems are associated with several uncultured methanotrophic bacteria, our study aimed at enrichment and isolation of novel methanotrophs from these environments.

Methods: Enrichment of aerobic methanotrophs started with materials collected from the Indian ocean hydrothermal vent fields (IHVF) and incubated at temperature range of 30-65°C. Several techniques were employed for the isolation and identification of the methanotrophs.

Results: Methane-oxidation was observed in cultures setup at 30°C and 40°C and were successively transferred to fresh media. The abundant methanotrophs were designated as strains E3 and E4 respectively. The pmoA sequence of strain E4 showed 91.67% identity to that of *Methylothermus thermalis,* whereas strain E3 showed 95.30% identity to *Methylomarinum vadi* at the amino acid level. The closest relative for pmoA sequence of strain E4 is a partial pmoA sequence (98.61% identity at the amino acid level) of an environmental clone retrieved from deep-sea hydrothermal system. Our findings suggest that various methanotrophs in diverse phylogenetic clades are involved in CH_4 oxidation in hydrothermal vent systems. Likewise, temperature seems to be a key parameter affecting taxonomic profiles of methanotrophs. So far, every attempt to isolate strains E3 and E4 using solid media has failed. To get insight on CH_4 oxidation from IHVF, metagenomic sequencing for genome reconstruction and metabolic pathways of methanotrophs in the enrichment will be presented.

PW292 Effect of Be9, a microbial strain isolated from mining porewaters, on the biogeochemical cycle of uranium

Iván Sánchez-Castro[1], Pablo Martínez-Rodríguez[1], María Pinel-Cabello[1], Germán Bosch-Estévez[1], Michael Descostes[2], Mohamed Larbi Merroun[1]

[1]*University of Granada, Microbiology, Granada, Spain*
[2]*AREVA Mines, LR&D Department, Paris, France*

Background: Influence of microbial strains in the migration of heavy metals (HM) in natural environments is a process of great concern. A precise handling of certain microbes could assist in the precipitation/solubilization processes of valuable HM such as uranium. In particular, members of the genus *Microbacterium* are known to present high uranium tolerance due to multiple detoxication mechanisms. In a previous study, the *Microbacterium* strain Be9, isolated from porewaters of former mines, showed high tolerance to HM and a remarkable metabolic versatility.

Objectives: The ability of this isolate to interact with uranium has been evaluated to disentangle its role in the mobilization/immobilization of this radionuclide depending on physicochemical conditions.

Methods: For this purpose, we employed a multidisciplinary approach combining colorimetric methods for measuring inorganic phosphates release or uranium removal rates, microscopic techniques like STEM-HAADF, etc.

Results: *Microbacterium* sp. Be9 displayed different behaviours in contact with uranium depending on the incubation conditions. Experiments performed in a complex culture medium showed the ability of Be9 to prevent U abiotic precipitation. Moreover, when exposed to pre-formed abiotic uranium precipitates of complex nature, this isolate was able to completely re-solubilize it. By incubating Be9 and U in a model background electrolyte without phosphates, significant uranium removal occurred. However, when amending organic phosphates the removal rate decreased drastically. In conclusion, we demonstrated the highly variable behaviour of this bacterial strain when contacting it with uranium. Based on these results, this strain might be considered a promising candidate for uranium recovery after its precipitation in biofilters.

PW293 Consequences of continuous cropping on ginseng soil microbial community

Jerald Ibal[1], Byung Kwon Jung[1], GunSeok Park[1], Chang Eon Park[1], Jae-Ho Shin[1]

[1]*Kyungpook National University, Daegu, Korea, Rep. of South*

Background: The soil microbial community is widely known to play an important role in plant growth and health. The use of continuous monocropping is known for playing a large part in producing low crop yields caused by soil-borne pathogens. Although the *Panax ginseng,* used in oriental medicine, is still cultivated using continuous monocropping, little information is available about it's soil microbial community.

Objectives: The objectives of this study were to investigate and compare the soil microbial communities of healthy and unhealthy ginseng crops as well as to compare them with that of ginseng soil microbial communities from crop rotated fields.

Methods: Soil samples were collected in a time-series manner along with their physicochemical properties. Continuously cropped healthy and unhealthy ginseng soils and crop rotated soils (ginseng/bean and ginseng/rice) were collected from Sangju and Punggi, South Korea, respectively. DNA were extracted from the soil samples and were sequenced for their 16S rRNA genes using Ion torrent PGM.

Results: Distinct microbial communities were associated in the different cultivation methods. An increase in diversity of the bacterial communities present in the unhealthy ginseng soil in comparison with healthy ginseng soil and the crops cultivated in crop rotation (bean and rice) was observed. Bray-curtis dissimilarity showed distinction among the groups. Healthy ginseng soil samples tended to be closer to each other while unhealthy ginseng soil samples clustered together and soil samples from ginseng that were cultivated in crop rotation were found in a different cluster

PW293 arabidopsis thaliana induces salt tolerance and multi-generational growth promotion in bacillus subtilis

Omri Gilhar[1;1], Ilana Kolodkin-Gal[1]

[1]*weizmann institute of science, molecular genetics, rehovot, Israel*

Background: *B. subtilis* and other plant growth promoting bacteria emit a complex blend of volatile organic compounds (VOCs) that enhance plant growth and abiotic stress tolerance. However, it remains unknown whether plants alter bacterial response to high salinity.

Objectives: We so far demonstrated that plants confer multigenerational salt tolerance to the beneficial bacterium *B. subtilis*. Now we aim to test whether this interaction confirms increased fitness in the competitive rhizosphere microenviornment and to identify the mechanism behind the beneficial long-lasting effect of the plant.

Methods: *B. subtilis* bacteria were cultured in *A. thaliana* secretions and then washed. Planktonic growth of the bacteria with and without NaCl was monitored. Fitness of the bacteria in sand was assessed by measuring the culturability of the bacteria in the presence or absence of interaction with the plants. These bacteria were then grown for 30 generations and the salt tolerance of the descendants was examined in a similar fashion.

Results: *B. subtilis* bacteria pre-cultured with plant secretions exhibited a decreased lag time in non-saline conditions, and an even larger decrease in the presence of high salinity, as compared to bacteria not pre-cultured with plant secretions. Pre-culture in plant secretions also improved the bacterial fitness in sand in the absence and presence of high salinity, exhibiting again a more significant effect of plant secretion on fitness in saline sand. Furthermore, the descendants from at least 30 generations down the lineage of bacteria pre-cultured with plants maintained a decreased lag time.

PW294 Meta-omics investigation of soil communities along a natural climatic gradient in the Finnish Arctic tundra

Jenni Hultman[1], Igor Pessi[1], Sirja Viitamäki[1]

[1]*University of Helsinki, Department of Microbiology, Helsinki, Finland*

Background: Climate change is affecting the arctic dramatically as the warming is fastest in higher latitudes. Previously frozen ground is thawing and releasing substantial quantities of carbon which microbes can decompose. Microbes mineralize the carbon fraction and convert it to carbon dioxide and methane. Climate change models estimate that C released from thawing arctic permafrost can represent the largest future transfer of C from the biosphere to the atmosphere. In addition to temperature, also oxygen and moisture, among many others, affect the microbial activity.

Objectives: Understanding the drivers of arctic soil communities and especially how the microbial activity changes with warming and resulting changes in soil characteristics is needed. However, this kind of information in lacking for the arctic soil microbial communities.

Methods: We have analyzed over 100 soil plots from a large field site in Scandinavian low arctic with environmental gradient of microclimatic conditions. Metagenomic approach was used to create a database of microbial genes and metagenome assembled genomes (MAGs) found within this gradient. As DNA can originate from dead or dormant cells, we utilized RNA sequencing to create comprehensive understanding on the metabolic activities of microbes within this fine-scale climatic variation.

Results: The metabolic potential of the soil communities showed, for example, the importance of carbon, nitrogen and sulphur cycles in the tundra ecosystem. By closing critical knowledge gap through integration of microbial activity from meta-omics data to process and Earth system model development will increase our general understanding about microbial community function in the changing Arctic.

PW295 Antimicrobial activity of Chitinophaga sp. Mgbs1 isolated from a tropical peat swamp forest in Malaysia using a modified iChip

Calvin Bok Sun Goh[1], Lee Sui Mae Lee[1], Pooria Pasbakhsh Pasbakhsh[2], Cheow Yuen Lin Cheow[1], Philip Marriott Marriott[3]

[1]*Monash University Malaysia, School of Science, Subang Jaya, Malaysia*
[2]*Monash University Malaysia, School of Engineering, Subang Jaya, Malaysia*
[3]*Monash University / Wellington Road, Faculty of Science, Clayton, Australia*

Background: The extensive use of antimicrobial agents has led to the evolutionarily-driven rise of antimicrobial-resistance (AMR) worldwide which raises a complex global health challenge. Existing natural antibiotics have acted as molecular scaffolds for chemical tailoring that has fulfilled the needs of clinical settings for the past five decades. Today, compound rediscovery is considerable due to the difficulty in culturing a great proportion of microbial taxon *in vitro*.

Objectives: The present study aims to cultivate environmental microbes from a pristine tropical peat swamp in Malaysia and to isolate the respective antimicrobial compounds from those with antimicrobial activity.

Methods: An isolation chip for *in situ* microbial cultivation was used within peat matter. The bacterium with the broadest antimicrobial spectrum_was selected for further bioactive compound isolation and characterization. The bacterium of interest was grown in its optimal broth medium in the presence of adsorbant amberlite XAD-16N. Adsorbed compounds on the amberlite were then harvested and subjected to sequential extractions. The active extract was determined via minimal inhibitory and bactericidal concentration against drug-resistant pathogens.

Results: A novel bacterium *Chitinophaga* strain Mgbs1 has been discovered that manifests a broad-spectrum antimicrobial activity. Lyophilized dichloromethane extracts of concentrated amberlite washings had significant activity against drug-resistant pathogens including *Acinetobacter_baumannii* ATCC_BAA1605, *Staphylococcus_aureus* ATCC_BAA 1717, and *Escherichia_coli* ATCC_BAA2523 with MIC values of 5.0, 10.0 and 78.0 µg/mL respectively. We believe that strain Mgbs1 produces a novel antimicrobial compound as no other *Chitinophaga* species produces antimicrobials with the same antimicrobial spectrum as that of Mgbs1.

PW296 Characterization of biofilms in an aquaponic system

Zala Schmautz[1;2], Andrea M. Bohny[2], Fabio Rezzonico[1], Nadine Antenen[1], Ranka Junge[1], Emmanuel Frossard[2], Theo H. M. Smits[1]

[1]*Institute of Natural Resource Sciences, Life Sciences and Facility Management, Zurich University of Applied Sciences, Wädenswil, Switzerland*
[2]*Institute of Agricultural Sciences, Department of Environmental Systems Science, ETH Zurich, Lindau, Switzerland*

Background: Microbial communities and their metabolites are essential for the functionality of aquaponic systems, serving as a connection between fish excrements and plant fertilization. However, just as fish and plants, the community structure also depends on specific abiotic conditions within the system.

Objectives: A characterization of the bacterial and archaeal communities is needed to define their role in the physiological processes within aquaponic systems.

Methods: The microbial community structure was investigated with genetic fingerprinting (T-RFLP) in several compartments of the aquaponic system. This was complemented with monitoring of environmental water parameters including temperature, pH, redox potential, dissolved oxygen (DO), electrical conductivity (EC), total nitrogen (TN), organic carbon (TOC) and relevant anions and cations.

Results: The results indicate major differences between the compartments. Richness and diversity of the microbial species were higher in aerobic than in anaerobic environments. Furthermore, bacteria showed higher taxa richness, diversity and community evenness, while archaea showed higher β-diversity. The variation of microbial community structure could be explained through differences in the TN, TOC, redox potential, EC and DO. Additionally, a correlation between the presence of inorganic nitrogen compounds and the community structure was observed, implying that the fundamental differences in abiotic water parameters between compartments influence microbial communities and vice versa. To further link the physiological role of biofilms with the water quality, fish and plant health, a more thorough characterization of bacterial and archaeal species diversity, their functions, metabolism and nutrient conversions would be required.

PW297 Community composition and seasonal changes of archaea in coarse and fine particulate matter

Jörn Wehking[1], Daniel A. Pickersgill[1], Robert M. Bowers[2], David Teschner[1], Ulrich Pöschl[1], Janine Fröhlich-Nowoisky[1], Viviane R. Després[3]

[1]*Max Planck Institute for Chemistry, Multiphase Chemistry Department, Mainz, Germany*
[2]*DOE Joint Genome Institute, Walnut Creek, CA, United States*
[3]*Johannes Gutenberg University, Institute of Molecular Physiology, Mainz, Germany*

Background: Archaea are ubiquitous in terrestrial and marine environments and play an important role in biogeochemical cycles. Although air acts as the primary medium for their dispersal among different habitats, their diversity and abundance is not well characterized. The main reason for this lack of insight is that archaea are difficult to culture and low in number in the atmosphere

Objectives: To understand the transport, residence time, and living conditions of microorganisms as well as their effects on the atmosphere and vice versa, it is essential to study all groups of bioaerosols.

Methods: Here we present an in-depth analysis of airborne archaea based on Illumina sequencing of 16S rRNA genes from atmospheric coarse and fine particulate matter.

Results: We show seasonal dynamics and discuss anthropogenic influences on the diversity, composition and abundance of airborne archaea. The proportions of archaea to bacteria, differences of the community composition in fine and coarse particulate matter, and the high abundance in coarse matter of one typical soil related family, the Nitrososphaeraceae, point to local phyllosphere and soil habitats as primary emission sources of airborne archaea. We found comparable seasonal dynamics for the dominating Euryarchaeota classes and Crenarchaeota orders peaking in summer and fall. In contrast, we found the omnipresent Cenarchaeales and the Thermoplasmata occurring throughout summer and fall. We also gained novel insights into archaeal composition in fine particulate matter, with Cenarchaeaceae, Nitrososphaeraceae, Methanosarcinales, Thermoplasmata, and the genus *Nitrosopumilus* as dominating taxa. The seasonal dynamics of methanogenic Euryarchaeota point to anthropogenic activities as sources.

PW298 MicrobiomeSupport: Towards coordinated microbiome R&I activities in the food system to support (EU and) international bioeconomy goals

Tanja Kostic[1], Angela Sessitsch[1], MicrobiomeSupport Consortium[2]

[1]*Austrian Institute of Technology GmbH*
[2]*MicrobiomeSupport Consortium*

Background: Knowledge of the potential of microbiomes, throughout the food chains, is seen as a promising means to ensuring the sustainability of our food system. Although several relevant European programmes and initiatives are currently running or are being launched, they are largely fragmented, implying a stringent need for coordinated action. This need for joint action should also be regarded at the international level.

MicrobiomeSupport is a Coordination and Support Action uniting 27 partners with the overall objective to establish an international network of experts and stakeholders in the field of microbiome food systems research and assess applicability and impact of the microbiomes on the food system.

Objectives:

- **Identification and mapping** of microbiome activities, programmes and facilities along the food chain and beyond in the EU and worldwide
- **Creation of a platform** for scientists, regulatory experts, industry, funding and policy organisations as well as support of the International Bioeconomy Forum to implement the 'Food Systems Microbiome' working group
- **Improve use of existing data** to allow comparability and improved mining of microbiome data
- **Define strategic agendas** to enable joint international microbiome applications in the food sector and beyond
- **Collaboration and coordination** in support of a sustainable bioeconomy in Europe and worldwide, in line with the FOOD 2030 policy goals
- **Raising awareness and exchange of knowledge** across scientific and political communities, including the International Bioeconomy Forum and the public

Results:

The project started in October 2019 and its progress can be followed on www.microbiomesupport.eu or @MicrobiomeEU.

PW299 Metagenomic study of extremophiles from Andean lakes deepens the understanding of arsenic resistance and bioenergetics

Federico A. Vignale[1], Maria E. Farias[1], Adrian G. Turjanski[2], Alex L. Mitchell[3], Robert D. Finn[3]

[1]Planta Piloto de Procesos Industriales Microbiológicos (PROIMI), CCT, CONICET, San Miguel de Tucuman, Argentina
[2]Instituto de Química Biológica de la Facultad de Ciencias Exactas y Naturales (IQUIBICEN), Ciudad Autónoma de Buenos Aires, Argentina
[3]The European Bioinformatics Institute (EMBL-EBI), Hinxton, Cambridgeshire, United Kingdom

Background: Arsenic metabolism is proposed to be an ancient mechanism in microbial life. Both bacteria and archaea use detoxification processes to grow under high arsenic concentrations. Some bacteria are also able to use arsenic as a bioenergetic substrate, through either anaerobic arsenate reduction (*arrAB*), or aerobic arsenite oxidation (*aioBA*). However, archaeal bioenergetic arsenic metabolism has not yet been demonstrated.

Objectives: We performed metagenomic analyses of biofilms, microbial mats and microbialites from high altitude Andean lakes (HAAL), which present similar characteristics to primitive Earth (low oxygen pressure, high UV radiation, hypersaline alkaline waters and elevated volcanic origin arsenic).

Methods: Following shotgun sequencing of DNA extracted from the samples, taxonomic and functional annotation was performed using the MGnify metagenomic analysis pipeline. In addition, samples were assembled and binned using MetaSPAdes and MetaWRAP, respectively. Functional annotation of metagenomic bins was carried out with InterProScan, followed by assignment of functional attributes with Genome Properties. Arsenic resistance and bioenergetic genes were identified using profile hidden Markov models, and the abundance of these genes was determined by mapping reads to the binned genomes.

Results: The analyses revealed a high abundance of arsenic bioenergetic genes in Diamante Lake, which is dominated by haloarchaea, suggesting that these haloarchaea use arsenic compounds as bioenergy substrates or use *arrAB* and *aioBA* for detoxification. Moreover, the organization of operons and abundance of arsenic resistance genes shed light on the main mechanisms involved in arsenic detoxification within HAAL, which may have also been used by microorganisms in the early evolution of life.

PW300 Deciphering maize selection of plant beneficial bacteria via an experimental evolution approach

Beatriz Manriquez[1], Daniel Muller[1], Claire Prigent-Combaret[1]

[1]*Laboratoire d'Ecologie Microbienne UMR5557, Université Claude Bernard Lyon 1, Lyon, France*

Background: The rhizosphere hosts a huge diversified microbial community, including bacteria able to interact with plants, by means of direct or indirect plant beneficial properties; these bacteria are known as plant growth-promoting rhizobacteria (PGPR). The interactions between these partners influence plant development and physiology.

Objectives: Our work aims to prove that plants will select a PGPR assembly with plant beneficial properties that will maximize its beneficial effects on the plant. To support this hypothesis, we conducted an *in vitro* experimental evolution on maize plants with a PGPR assembly composed of 10 fluorescent *Pseudomonas* ("ancestral" assembly), half of them with a high number of plant beneficial properties (8 or more) and the other half with a lower number (6 or less).

Methods: Experimental evolution consists on the inoculation of the "ancestral" assembly on the plants and after 7 days of growth, bacterial assemblies retrieved from the roots are used as new inoculums for a fresh batch of plants. The experiment was carried out over 6 months.

Results: The expected results are (i) that plants would select PGPR with few plant beneficial properties and (ii) that there is coevolution between the PGPR within the assembly, to optimize plant beneficial effects, leading to "evolved" assemblies with fewer PGPR and with changes in their genomes. In the second phase, we would like to compare the plant beneficial effects between the "ancestral" and the "evolved" assemblies on two different maize genotypes and in presence or absence of a fungal pathogen *F. graminearum* (soil pot assays).

PW301 Horizontal gene transfer of antimicrobial resistance genes in British agricultural soils

Marcela Hernandez Garcia[1], Dorothee Kinkel[2], Marc Dumont[1], Charles W. Keevil[1]

[1]*University of Southampton*
[2]*University of Bonn*

Background: Horizontal gene transfer (HGT) confers resistance to many classes of antimicrobials and has resulted in a worldwide epidemic of nosocomial and community infections caused by multidrug-resistant microorganisms leading to suggestions we are returning to the pre- antibiotic era. Agricultural soils are a potential source of antimicrobial resistance (AMR) due to the ingress of antimicrobials and resistant microbes into soil.

Objectives: In this study we investigated the potential for horizontal gene transfer (HGT) in British agricultural soils.

Methods: Microcosms were established using three agricultural soils with different soil properties. These microcosms were inoculated with the donors *Klebsiella pneumoniae* NCTC 13443 (carrying $bla_{\text{NDM-1}}$ and $bla_{\text{CTX-M-15}}$) and *Escherichia coli* NCTC 13441 (carrying $bla_{\text{CTX-M-15}}$) together with a recipient *Escherichia coli* J53 (J53 AzR (F- *met pro*)) and incubated at room temperature for up to 48 h.

Results: The results showed that bacteria could be recovered on selective agar plates from the soil after 48 h, indicating that both the donors and recipient can survive in the soil. HGT of $bla_{\text{CTX-M-15}}$ from *K. pneumoniae* NCTC 13443 and *E. coli* NCTC 13441 to *E. coli* J53 occurred in soil. No conjugation of the plasmid containing the NDM-1 metallo-β-lactamase was observed. This study confirms that HGT can occur in the soil and there is the potential for dissemination of AMR genes in agricultural soils.

PW302 What's going on in hospital biofilm?

Bricheux Geneviève[1], Hilpert Cécile[1], Donnadieu Florence[1], Hennequin Claire[1], Christiane Forestier[1], Traore Ousmane[1], Debroas Didier[1]

[1]*Université Clermont Auvergne, LMGE, Aubière cedex, France*

Biofilm is the dominating bacterial lifestyle at the planet scale conferring recalcitrance and persistence properties to their inhabitants. The proximity of cells within biofilm promotes DNA exchanges, and the concomitant presence of antibiotics favors selection of resistant strains. Such criteria fit with hospital effluents, heavily loaded with bacteria and medical drugs.

In this study, the "transmission potential" of biofilm present in the outlet pipes of the hospital Gabriel Montpied (France) was investigated

Biofilms were formed on glass coupons settled in the flow of the outlet water for 3 weeks. After scraping and disorganization, a plasmid DNA enriched fraction was extracted using an alkaline lysis method. The DNA was then sequenced by Illumina generating more than $107x10^6$ reads.

Taxonomy analysis showed mainly the presence of Proteobacteria. The sequences associated with antibiotic resistance mechanisms were mainly efflux pump (*EmrE*), ABC transporter and beta-lactamase-encoding genes. Plasmid reconstitution was performed using a specific homemade pipeline. Its reliability was confirmed by searching for plasmid markers such as MOB, MPF, *OriT* and rep sequences. A total of 9490 linear plasmids and 381 circular plasmids (size 1.4 kb to 235 kb) were reconstructed, with 2 of them already in databases. The findings indicate that antibiotics producing bacteria are common in these biofilms and may disseminate the multiple antibiotic resistance traits among bacterial community through genetic exchange mechanisms. This study highlights the major potential role of biofilms in hospital effluents as providers of resistance genes to urban effluents and pose a hazard to environmental and public health.

PW303 Massive biofilm of iron bacteria in a cave with the remnants of military ordnance

Janez Mulec[1], Adrijan Košir[2]

[1]Research Centre of the Slovenian Academy of Sciences and Arts, Karst Research Institute, Postojna, Slovenia
[2]Research Centre of the Slovenian Academy of Sciences and Arts, Ivan Rakovec Institute of Palaeontology, Ljubljana, Slovenia

Background: The Yugoslav People's Army used the cave entrance of Matijeva jama in Slovenia till 1991 as a target to shoot with heavy artillery. Metal parts of exploded and unexploded ordnance mixed with rock and organic debris form a large cone on the bottom of the entrance shaft.

Objectives: In 2016 a brownish biofilm of approximately 7 m^2 was found extending from the bottom of the cone deeper into the cave. Fresh biofilm was 0.2 to 2 mm thick, predominantly of organic and mineralized tubular forms with a diameter of 1-2 µm and length of some 10 µm; spherical forms in diameter of about 3 µm were rare.

Methods: Iron minerals (15-60%) dominated the outer and inner layers of the biofilm, and in between some 10 µm thick layer expressed an increased presence of manganese (> 15%). Biofilm (pH ~ 7.5) showed a considerable amount of cultivable microbial biomass (~ 8.5×10^8 CFU/ g of dry matter under aerobic growth conditions, and 3.0×10^8 CFU / g of dry matter under anaerobic growth conditions at 10°C).

Results: Biofilm was dominated by Proteobacteria (76.3%) and Bacteroidetes (14.2%). The largest part of the identified sequences belonged to *Leptothrix* (25.0%), *Geobacter* (15.6%), *Rhodoferax* (5.3%), and *Anaeromyxobacter* (5.3%), which are associated with metal cycling in nature. More research is needed to understand oxido-reduction reactions in karst underground ecosystems, including in cases of remnants of exploded and unexploded ordnance because microbes can cause corrosive effects and leaching of dangerous substances into groundwater.

PW304 A non-sporulating variant of Paenibacillus polymyxa E681 with improved motility and energy production

Younmi Lee[1], Young Soo Kim[2], Youngsoo Seo[3], Choong-Min Ryu[4;5], Seung-Hwan Park[4;5], Yongho Jeon[6]

[1]Agricultural Science and Technology Research Institute, Plant medicine, Andong, Korea, Rep. of South
[2]Andong National University, Plant medicine, Andong, Korea, Rep. of South
[3]Pusan National University, Microbiology, Pusan, Korea, Rep. of South
[4]Korea Research Institute of Bioscicence and Biotechnology, Infectious disease Research Center, Daejeon, Korea, Rep. of South
[5]KRIBB School of Biotechnology, Korea University of Science and Technology, Biosystems and Bioengineering, Daejeon, Korea, Rep. of South
[2]Andong National University, Plant medicine, Andong, Korea, Rep. of South

Background: *Paenibacillus polymyxa*, a root-associated plant growth-promoting rhizobacterium (PGPR), has many beneficial effects on crop plants. Phenotypic variation of E681 occurs naturally and the characteristics of the wild-type 'B' and variant 'F' are very different in terms of spore formation, motility, and antibiotic production ability, among other things.

Objectives: We studied to better understand the physiological changes underlying this phenotypic variation

Methods: We employed an RNA-seq approach to compare genome wide patterns of gene expression and a comparative proteome analysis.

Results: We identified and analyzed the functions of 1,062 genes differentially expressed between the two phenotypic variants. Comparative RNA sequencing revealed that 'F' differed in many cellular functions including sporulation, flagella synthesis, carbohydrate metabolism, environmental information processing and antimicrobial compound production. Interestingly, the sporulation process was regulated at stage 0 in 'F'. Comparative proteome analysis has shown that 47% of the differentially expressed proteins were involved in glycolysis and other metabolic pathways associated with carbohydrate metabolism, and the rests are involved in flagella assembly, stress resistance and coenzyme production. Our results indicate that E681 exhibits at least two physiological responses to unfavorable environmental conditions: the first is an ability to form spores that are highly resistant to harsh conditions, and the second is transformation into the 'F' variant, which is characterized by increased motility and more active membrane transport systems driven by the energy saved by avoiding sporulation.

PW305 Characterization and genomic reconstruction of highly enriched methanotrophs, Methylocystis sp. B8

So-Jeong Kim[1], Gi-Yong Jung[1]

[1]Korea Institute of Geoscience and Mineral Resources, Geologic Environment Research Division, Daejeon, Korea, Rep. of South

Background: Methane is one of the green gases and its concentration has increased every year. Methane oxidizing bacteria are crucial players in capturing methane.

Objectives: To get an insight into novel genomic feature of methanotroph, we analyzed the draft genome sequence of *Methylocystis* sp. B8. from wetland.

Methods: The physiological tests were performed with enriched strain B8. Also, we reconstructed draft genome of methanotroph using metagenomic approaches.

Results: Based on 16S rRNA gene, strain B8 is close to *Methylocystis hirsuta* CSC1 (98% similarity) belonging to type II methanotroph. Average nucleotide identity was 84.1% between *M. hirsuta* CSC1 and strain B8. Strain B8 grew at 15-35 °C (optimum 30 °C). Cells of strain B8 were 1.3 µm wide and 2.7-2.9 µm in length. Colony was not formed. The genome was 3.39 Mbp and 59.9 mol% G+C content. Interestingly, this genome encoded two type of particulate methane monooxynagese(MMO), pMMO1 (the low-affinity methane oxidation enzyme) and pMMO2 (the high-affinity methane oxidation enzyme) and additional 2 copies of pmoC genes. The pMMO2 might allow strain B8 to live in low-methane environment. Serine, TCA and ethylmalonyl-CoA (EMC) pathway was completely encoded. Genes related to heavy metal detoxification were contained in the genome (arsenate reductase and mercury reductase). The presence of Ni/Fe-hydrogenase I (hyaAB) and hydrogenase nickel incorporation and accessory protein (hypAB) in this genome might suggest that hydrogen play roles as energy source in this strain. This unique genome makes strain B8 well adapted to various natural environment. Supported by KIGAM(19-3413).

PW306 Isolation and characterization of diesel and BTEX- degrading bacteria isolated from Arctic soil at low temperature

JiGwan Son[1]

[1]*Seoul National University, College of Agriculture and Life Sciences, Seoul, Korea, Rep. of South*

Background: In the recent years, widespread consumption of petroleum in cold climate area has led to soil pollution at cold site. Petroleum hydrocarbon contamination has received significant attention because the compound has harmful effects in human. Compared to physical and chemical methods, bioremediation has been considered to be more effective and less expensive option that causes less damage to cold climate environment in removing petroleum products.

Objectives: The aim of this study is to isolate, identify and characterize native bacterial strains from Arctic soil that could degrade petroleum products at low temperature.

Methods: Diesel-degrading bacterium was isolated from the Arctic soil using enrichment culture technique. The isolates were identified by 16S rRNA gene sequence analysis. Growth profiles were assessed by measuring the absorbance at wavelength 600nm on different temperature (4, 10, 18, and 28 ℃) on mineral medium broth. Diesel and BTEX- degrading ability was analyzed by GC-FID and GC-MS.

Results: 9 diesel-degrading bacteria species isolated from Arctic soil were identified as *Pseudomonas* sp.*, Pseudarthrobacter* sp.*, Arthrobacter* sp.*, Streptomyces* sp.*, Massilia* sp. These bacteria species utilized diesel as a sole carbon source for their growth. 3 species were characterized by maximal activity at 4℃, 5 species at 10℃ and 1 species at 18℃. 9 bacteria species utilized 83.1-50.5% of total diesel (200mg/L^{-1}) in 15 days of incubation at their optimum growth temperature. *Pseudarthrobacter siccitolerans*, the most efficient species in degrading diesel, was able to degrade all six BTEX components when BTEX was supplied as an individual component.

PW307 The limit of the genetic adaptation to herbicides in freshwater phytoplankton and the adaptation photosynthetic cost

Ignacio José Melero-Jiménez[1], Elena Martín-Clemente[1], Elena Bañares-España[1], Andreas Reul[2], María Jesús García-Sánchez[1], Antonio Flores-Moya[2]

[1]*Universidad de Málaga, Departamento de Biología Vegetal, Málaga, Spain*
[2]*Universidad de Málaga, Departamento de Ecología y Geología, Málaga, Spain*

Background: One of the most important anthropogenic impacts on freshwater aquatic ecosystems is the continuous increase of herbicide concentrations, which impacts on the structure of phytoplankton communities.

Objectives: The maximum adaptation of two green microalgae (*Dictyosphaerium chlorelloides* and *Chlamydomonas reinhardtii*) and a cyanobacterium (*Microcystis aeruginosa*) to two widely used herbicides: glyphosate (N-(phosphonomethyl)glycine) and diuron ($C_9H_{10}C_{l2}N_2O$, 3-(3,4-diclorofenil)-1,1-dimetilurea) was explored. Additionally, the cost of the herbicide resistance on photosynthesis and growth rate was evaluated.

Methods: We used an eco-evolutionary approach (ratchet protocol) to explore the maximum genetic adaptation. We characterised the photosynthetic performance by oxygen production and PSII chlorophyll *a* fluorescence.

Results: A dose of 1 µM diuron or 40 ppm glyphosate completely inhibited the growth of *M. aeruginosa and D. chlorelloides*, whereas *C. reinhardtii* growth was completely abolished at 2 µM diuron or 90 ppm glyphosate. However, an increase in resistance to both herbicides was achieved in certain populations during the ratchet experiment. *Microcystis aeruginosa and D. chlorelloides* were able to adapt up to 8 µM diuron and 80 ppm glyphosate, whereas *C. reinhardtii* adapted up to twice these herbicide concentrations. The photosynthetic performance was generally lower in the resistant than in the wild-type strains in the three species. These results suggest that increasing concentrations of these herbicides in freshwater bodies could induce the selection of herbicide-resistant mutants in phytoplankton communities but showing lower primary production than original populations.

PW308 Proteome analysis of Streptomyces scabies grown in presence of potato

Lauriane Giroux[1], Iauhenia Isayenka[1], Nathalie Beaudoin[1], Carole Beaulieu[1]

[1]*Université de Sherbrooke, Biologie*

Background: *Streptomyces scabies* is the major causal agent of potato common scab. The disease is characterized by the presence of suberized scab lesion on tubers. Phytotoxins called thaxtomins are known to be the only pathogenicity factors. However *S. scabies* can also produce other metabolites such as coronafacic acid and concanamycins.

Objectives: As the interactions between *S. scabies* and potatoes are not well characterized, proteomics studies aiming to identify proteins produced during these interactions were carried out.

Methods: Potato microtubers from the resistant cultivar Russet Burbank (RB) and the sensitive cultivar Yukon Gold (YG) were produced on Murashige-Skoog medium with 8% sucrose. *S. scabies* was then grown in a liquid growth medium in the absence or presence of 6-week-old or 6-month-old potato microtubers of each cultivar.

Results: Proteins linked to the production of concanamycins and the siderophore pyochelin were detected in the presence of tubers only. These proteins were found more abundant when the bacterium was grown with the resistant cultivar than the sensitive one with both young and old microtubers. Proteins involved in different stress responses (SOS response, DNA repair, oxidative stress) were also found to be more abundant in the presence of microtubers than when the bacterium was grown alone, which suggests a bacterial stress adaptation during host infection

PW309 Sulphide resistance in the cyanobacteria Oscillatoria sp. and Microcystis aeruginosa: a comparative study of photosyntetic performance between wild-type and mutants with a higher sulphide tolerance

Elena Martín-Clemente[1], Ignacio José Melero-Jiménez[1], Elena Bañares-España[1], Andreas Reul[2], Antonio Flores-Moya[1], María Jesús García-Sánchez[1]

[1]*Universidad de Málaga, Departamento de Biología Vegetal, Málaga, Spain*
[2]*Universidad de Málaga, Departamento de Ecología y Geología, Málaga, Spain*

Background: Cyanobacteria show different degrees of tolerance to sulphide. An *Oscillatoria* sp. strain, isolated from a sulphureous spa, grows until 0.9 mM sulphide, whereas *M. aeruginosa* is sensitive to sulphide concentrations below 0.1 mM. Applying an experimental evolutionary approach, we obtained a *M. aeruginosa* strain adapted to 0.4 mM sulphide and an *Oscillatoria* mutant tolerant to 2 mM sulphide.

Objectives: The photosynthetic performance of wild-type and mutant strains was explored to shed light about the process of sulphide adaptation and tolerance in cyanobacteria. The physiological cost of the mutation conferring sulphide resistance was also explored.

Methods: Oxygen evolution and growth rate in the absence of sulphide, plus pigment content and cell morphology was compared between wild-type and mutant strains of each species. The photosynthetic performance at increasing sulphide concentrations was characterized in all strains, measuring maximum quantum yield of PS II (F_v/F_m) and electron transport rate (ETR). Finally, we measured ^{14}C fixation to detect the possible existence of anaerobic sulphide-dependent photosynthesis.

Results: We observed that sulphide adaptation leads to a decrease in growth and photosynthetic rates in the absence of sulphide, to lower Chl *a* content and different morphology in mutant strains. As expected, *Oscillatoria* maintained higher F_v/F_m values than *M. aeruginosa* at high sulphide concentrations. Nevertheless, F_v/F_m and ETR in the presence of sulphide were higher in both mutant strains compared to wild-type ones. However, none of the strains showed sulphide-dependant anoxygenic photosynthesis. Thus, sulphide tolerance in *Oscillatoria*, could be related to an enhanced PS II resistance to sulphide.

PW310 High content analysis of microbiota on medicinal properties possessing berries

Juliana Luksa[1], Iglė Vepštaitė-Monstavičė[1], Ramunė Stanevičienė[1], Živilė Strazdaitė-Žielienė[1], Saulius Serva[2], Elena Servienė[1]

[1]*Nature Research Centre, Laboratory of Genetics, Vilnius , Lithuania*
[2]*Vilnius University, Department of Biochemistry and Molecular Biology, Vilnius , Lithuania*

Background: The quality of fruits and berries along with the content of active components depend on the cultivation and climatic conditions during vegetation, application of agrochemicals, hydration and harvest time. Microorganisms colonizing the surface of fruits, leaves, stems or living within tissues have a major influence on plant development, adaptation and evolution, in turn affecting plant potential in food production. Moreover, plant-associated microorganisms may cause foodborne diseases or may have relevant effect for human health by contributing to the diversity within gut microbiome or by stimulating immune response. The high potential of sea buckthorn, black chokeberry, red and white currants in healthy food industry boosted interest in the plant cultivation. The present study is the first work providing comprehensive information on microbial populations of these berries.

Objectives: The goal of the current study was to identify the composition of bacterial and fungal microorganisms associated with the black chokeberry, sea buckthorn, red and white currants harvested in Lithuania.

Methods: Metagenomics analysis using Next Generation Sequencing.

Results: Differences in diversity, composition and overall prevalence of eukaryotic and prokaryotic microorganism were dependent on the host plant. Among prokaryotic and eukaryotic consortia, we have identified potentially beneficial and pathogenic microorganisms. Obtained data substantiate understanding of the interactions between resident microflora and plant. The study uncovers the importance of microbiota in berries-based food production and deepens knowledge of their ecological and medical potential.

PW311 Yeasts-commensals in the sea buckthorn ecosystem

Ramune Staneviciene[1], Juliana Lukša[1], Iglė Vepštaitė-Monstavičė[1], Živilė Strazdaitė-Žielienė[1], Elena Servienė[1]

[1]*Nature Research Centre, Laboratory of Genetics, Vilnius , Lithuania*

Background: Plants host many yeast species that may be transported from the soil by wind, insects and animals or could be permanent residents on fruits, employing them as depositories for survival and propagation. The plant-associated yeast community year-to-year is characterized by the appearance of many new patterns, related to plant ripening stage, climatic conditions and other factors. Such microorganisms may be beneficial, inducing resistance in the hosting plant or pathogenic, responsible for disease development. Yeasts have been reported as commensal organisms of insects not only for attraction to food but also for the insect's development and behavior. Sea buckthorn is a popular garden and landscaping plant, native to Europe and Asia with high potential in control of environment, food industry and medicine. The present study for the first time reveals the structure of yeast communities associated with the sea buckthorn berries at different ripening stages.

Objectives: The goal of the current study was to identify yeast population inhabiting the sea buckthorn berries collected during ripening period.

Methods: Culture-dependent approaches and amplicon-based next-generation sequencing (NGS).

Results: Based on microbiological analysis, restriction fragment length polymorphism (RFLP) and sequencing of the 18S rRNA ITS1/ITS2 domain data, cultivable yeast species were determined. Whole sea buckthorn-associated yeast community was investigated employing NGS approach. Analysis of representative OTUs showed a clear separation among yeasts inhabiting the unripen and ripen berries. Differences in the structure of yeast populations uncover specific interactions between yeast and hosting plant as well plant-related insects.

PW312 Soil microorganisms in natural and managed forests of beech and Norway spruce

Richard Gere[1], Erika Gömöryová[1], Mikuláš Kočiš[1], Marián Homolák[1]

[1]*Technical University in Zvolen, Department of Natural Environment, Zvolen, Slovakia*

Microorganisms, as a part of the forest soil ecosystem, are subject to an ever-changing complex of soil properties driven by changes in microclimate and the input of organic matter and exudates, released by dominating tree species. Such an effect can differ in natural and managed forest stands.

The objective was to evaluate the effect of managed stands of *Fagus sylvatica* and *Picea abies*, and natural mixed stands of both tree species on microbial characteristics within the forest floor and two soil depths.

Microbial biomass, activity (measured by catalase activity, N-mineralization and basal respiration) and diversity of functional groups based on Biolog method were studied at 103 plots in Poľana Mts. (Slovakia). Analysis of variance (ANOVA), Tukey´s HSD tests and Canonical Correspondence Analysis (CCA) were used for statistical interpretation of aquired data.

Nearly all microbial indicators (except microbial carbon) differed significantly between forest stands, whether it was within the forest floor or mineral horizon. While within the forest floor, especially the managed spruce stands differed in microbial attributes from the other stands, in mineral horizon, the natural forests differed significantly from the managed forests. The differences between the forest floor and mineral horizon, as well as both depths of mineral horizon were also found.

PW313 Yeast community associated with Alvarinho wine grapes from different geographic regions

Paulo Fernandes[1], Jéssica Pereira[2], Isabel Afonso[3], Júlio Lopes[3], Ana Sofia Rodrigues[3]

[1]Viana do Castelo, Centro de Investigação e Desenvolvimento em Sistemas Agroalimentares e Sustentabilidade (CISAS), Instituto Politécnico de Viana do Castelo, ESTG-IPVC, Viana do Castelo, Portugal
[2]Ponte de Lima, 2 Instituto Politécnico de Viana do Castelo, ESA-IPVC, Ponte de Lima, Portugal
[3]Ponte de Lima, Centro de Investigação e Desenvolvimento em Sistemas Agroalimentares e Sustentabilidade (CISAS), Instituto Politécnico de Viana do Castelo, ESA-IPVC, Ponte de Lima, Portugal

Background: As revealed in recent studies, microbial flora associated with vineyards is affected and influenced by numerous factors related to climate and soil attributes, presenting some distribution patterns that in some cases can be geographic related.

Objectives: The aim of the present work was to determine the yeast community on the surface of Alvarinho wine grapes, in the 2018 harvest, from distinct geographic locations across Portugal, namely sub-regions of Monção&Melgaço, Basto, Lima and Cávado (Vinhos Verdes Region), Chaves (Trás-os-Montes Region), Terras de Azurara (Dão Region) and Vidigueira (Alentejo Region) using a combined approach of culture dependent and independent methods.

Methods: Microbial flora associated with the surface of Alvarinho wine grapes was extracted by washing and sonication. The suspension obtained was used to isolate colonies on selective culture media for yeasts. Colonies obtained were screened by PCR-RFLP, and phenotypically identified using Biolog and/or by sequencing of the ITS region. The suspension obtained was also used to obtain total DNA, and, high-throughput sequencing of the fungal ITS2 region was performed.

Results: Illumina sequencing resulted in 418 975 valid reads corresponding to a richness of 754 fungal species. High diversity could be found in all the parcels being *Sporidiobolaceae* the most abundant family (23%) present in all the parcels analysed. Fungal species richness is lower in the sub-region of Monção & Melgaço when compared to the other regions/sub-regions. Using culture dependent methods, a total of 127 isolates were phenotypically and/or genotipically identified and its prevalence correlated with each of the regions/sub-regions.

PW314 Foodborne pathogens isolated from livestock farms and enviroments

Hyeri Kim[1], Jae Hyoung Cho[1], Sun Hee Lee[1], Robin Guevarra[1], Jun Hyung Lee[1], Jin Ho Cho[2], Minho Song[3], Hyeun Bum Kim[1]

[1]Dankook University Cheonan Campus, Animal Resources Science, Cheonan, Korea, Rep. of South
[2]Chungbuk National University Gasin Campus, Division of Food and Animal Sciences, Cheongju, Korea, Rep. of South
[3]Chungnam National University, Division of Animal and Dairy Science, Daejeon, Korea, Rep. of South

Background: The consumption of foodborne pathogens contaminated on products from animals has been considered as a major food safety issue wordwide. Foodborne pathogen contamination on animal food products may originate at the farm level.

Objectives: This study was conducted to evaluate the prevalence of 9 foodborne pathogens including *Escherichia coli*, *Salmonella* spp., *Bacillus cereus*, *Staphylococcus aureus*, *Clostridium perfringens*, *Campylobacter* spp., *Listeria monocytogenes*, *Shigella* spp., and *Yersinia enterocolitica* in chicken livestock animal farm environments in South Korea.

Methods: Samples were collected from a range of environments common to most animal farms (5 beef cattle, 2 swine and 1 chicken farm) such as water, feed and soil sources as well as animal feces were cultured using selective agar media. The 16S rRNA gene sequencing and basic local alignment search tool (BLAST) were used to confirm the identity of the bacterial isolates.

Results: A total of 300 isolates were taken from farm environments and animal feces of which 160 isolates were identified as foodborne pathogens. *E. coli*, *Salmonella enterica* and *Clostridium perfringens* were the most prevalent among the foodborne pathogens tested from all the samples. All pathogens tested were identified in all the samples regardless of the farms. However, foodborne pathogens detected from swine farms higher than chicken and beef cattle farms. Moreover, fecal samples showed the highest diversity of pathogens, containing 9 of the 9 pathogens tested. Monitoring contamination levels of food borne pathogens may help control the spread of foodborne disease.

PW315 Dynamics of Molluscan Vibrio species sourced to Canadian Estuaries: Concerns and Probable Benefits to Human Health

Swapan K. Banerjee[1], Januana S. Teixeira[1], Meghan A. Biggar[1], Jeff M. Bussey[1]

[1]*Bureau of Microbial Hazards, Food Directorate, Health Canada, Canada*

Background: *Vibrio* species are indigenous to the estuarine environments around the world and implicated in human health concerns associated with water- and seafood-borne illnesses. Genetic adaptation enables *Vibrio* spp to survive environmental challenges from biotic and abiotic parameters.

Objectives: This study aimed at finding the impact of climate change on the composition and dynamics of *Vibrio* species in the Canadian estuaries as detected in molluscan shellfish from selected harvest sites of Atlantic Canada.

Methods: *Vibrio* species, particularly *V. parahaemolyticus* (Vp), *V. vulnificus* (Vv), *V. fluvialis* (Vf), *V. alginolyticus* (Va) and *V. cholerae* (Vc), were isolated from molluscs (oysters, clams and mussels) sourced to Atlantic estuaries of Canada, between May and October, 2014 to 2018. Biochemical and molecular assays, including polymerase chain reaction (PCR), were used to identify and characterize the isolates.

Results: During the five year period, 103 out of 110 (94%) harvested samples of bivalve molluscs tested positive for *Vibrio* species. Frequency of detection (Fd) of the four main *Vibrio* species were: *V. alginolyticus* (Fd= 81%), *V. parahaemolyticus* (Fd= 66%), *V. vulnificus* (Fd= 18%) and *V. cholerae* (Fd= 17%). In addition, *V. fluvialis* (Fd= 5%) and *V. mimicus* (Fd= 5%) were sometimes detected.

Conclusions: This study provided evidence of a trend in the composition of *Vibrio* species probably impacted by climate change. Tropically abundant *V. cholerae* is emerging in temperate Canada. Known to be mostly non-pathogenic, *V. alginolyticus* is acquiring clinical significance after being identified from Canadian patients in recent years.

PW316 Host Dependency of an Antarctic DPANN Archaeon

Joshua Hamm[1], Susanne Erdmann[2], Emiley A. Eloe-Fadrosh[3], Timothy J. Williams[1], Michelle A. Allen[1], Ricardo Cavicchioli[1]

[1]*UNSW Sydney, Biotechnology and Biomolecular Sciences, Kensington, Australia*
[2]*Max Planck Institute for Marine Microbiology, Bremen, Germany*
[3]*Joint Genome Institute, Walnut Creek, United States*

Background: Recent genomic interrogation of the domain Archaea has identified a number of novel, uncultivated lineages that possess broad ecological and evolutionary significance. The DPANN superphylum represents a globally distributed lineage characterised by reduced genomes, small cell sizes, and in some cases, a symbiotic lifestyle. Cultivation of these enigmatic Archaea has lagged the insights gained from sequence data, with only two major lineages reported to have been cultivated (Nanoarchaeota and Mircrarchaeota).

Objectives: To cultivate two Antarctic strains of a previously uncultivated DPANN lineage.

Methods: Enrichment cultures enabling the growth of the DPANN archaeon were established. DPANN cells were purified using fluorescence activated cell sorting for use in growth studies. Fluorescence in-situ hybridisation and transmission electron microscopy were used to assess cell structures and interactions; metagenomics to characterise enrichment taxa, generate metagenome assembled genomes and assess Antarctic communities; and proteomics to assess metabolic pathways and identify potentially relevant proteins.

Results: Growth of the DPANN archaeon alongside its host was established using FACS obtained cells. Microscopy revealed the intimate nature of the interaction including apparent membrane fusion and potential direct cytoplasmic transfer. Metabolisms were inferred from proteomics and potential metabolic interactions identified. Protein families possessing functional domains indicative of a role mediating interactions between the host and symbiont, present in multiple DPANN lineage metagenome assembled genomes derived from globally distributed datasets, were also identified. This work expands the range of archaeal symbioses and provides an additional model system for DPANN-host interactions to advance understanding of the factors controlling microbial symbiotic relationships.

PW317 Microbiota associated to the blooming of an invasive diatom (D. geminata) in ecosystems of chilean Patagonia

Karla Martinez-Cruz[1], Maialen Barret[2], Armando Sepúlveda Jáuregui[3], María Soledad Astorga España[3]

[1]*University of Magallanes, Sciences Faculty, Punta Arenas, Chile*
[2]*École Nationale Supérieure Agronomique de Toulouse, Auzeville-Tolosane, France*
[3]*University of Magallanes, Punta Arenas, Chile*

Background: The diatom *Didymosphenia geminata* has emerged as nuisance species in freshwater ecosystems around the world, developing thick mats of carbohydrate stalk material in ultraoligotrophic streams and lakes. *D. geminata* mats are nutrient-rich environments, that serve as substrate for a wide range of microorganisms capable of influencing biogeochemical cycles in their ecosystem. Therefore, analyzing the microbiota associated to *D. geminata* mats represents a pivot tool in our understanding of this invasive diatom's role in the biogeochemical cycles of affected lakes and rivers.

Objectives: To determine bacteria and archaea attached to *D. geminata* mats from three different ecosystems located in the chilean Patagonia.

Methods: 24 samples of *D. geminata* mats, attached to rocks, were collected during the spring of 2017 from three ecosystems located in the chilean Patagonia. Total DNA of samples was processed to sequence 16S rRNA genes using Illumina MiSeq technology.

Results: Alpha diversity showed that *D. germinata* mats in Serrano River were less diverse than those in Lake Blanco and Grande River. Archaea did not contribute significantly to the overall microbial communities. The core microbiome of *D. germinata* mats was defined based on the taxa shared among samples. These taxa are dominated by psychrophilic microorganisms, including organic carbon degrading bacteria and heavy metals tolerant bacteria. The dominant genera depended on environmental conditions and included potential N_2-fixing members, suggesting a possible association between *D. geminata* and N-fixers. This association could represent an important input of N into these ecosystems, with potential greater consequences on nutrient cycling than previously thought.

PW318 The ruminal microbiota adjusts to prevent acute acidosis onset in feed-withdrawn feedlot steers

Ana Rabaza[1], Georgget Banchero[2], Cecilia Cajarville[3], Pablo Zunino[4], Alejandro Britos[3], José Luis Repetto[5], Martín Fraga[1]

[1]*Instituto Nacional de Investigación Agropecuaria (INIA), Plataforma de Salud Animal, Colonia, Uruguay*
[2]*Instituto Nacional de Investigación Agropecuaria (INIA), Programa de Carne y Lana, Colonia, Uruguay*
[3]*Facultad de Veterinaria, Universidad de la República., Departamento de Nutrición Animal, San José, Uruguay*
[4]*Instituto de Investigaciones Biológicas Clemente Estable, Departamento de Microbiología, Montevideo, Uruguay*
[5]*Facultad de Veterinaria, Universidad de la República., Departamento de Producción de Bovinos, San José, Uruguay*

Background: Feed withdrawal is a common issue in open outdoor feedlot systems, where unpredicted circumstances can difficult the animals' access to food. The relationship among feed restriction, ruminal chemistry and microbiota, and acidosis onset has not been completely explained. This study hypothesized that feed withdrawal followed by feed reintroduction (**FW-FR**) may raise the risk of acidosis in grain-adapted cattle.

Objectives: The FW-FR model is explored to mimic field situations in which the access to the bunk feeder is restricted.

Methods: Twenty ruminally catheterized steers feeding a high-concentrate diet (85% of concentrate) were challenged by the FW-FR protocol. Animals were randomly assigned to: feed withdrawal for 12h (**T12**), 24h (**T24**), 36h (**T36**) or no feed withdrawal (control-**CON**) followed by the feed reintroduction. The ruminal chemistry, ruminal microbial community and clinical symptomatology of acute acidosis (**AA**) were assessed.

Results: The FW-FR challenge did not induce AA even though animals from the T36 treatment presented ruminal pH values consistent with this metabolic disease. Ruminal lactic acid accumulation in steers from T24 and T36 probably led to the stepwise decrease of pH in these groups. Twelve hours post-feed reintroduction, the feed-withdrawn animals presented a significantly increased relative abundance of Firmicutes whereas Bacteroidetes remained the dominant in the CON steers (P<0.05). Whereas fibrolytic bacterial groups (*Ruminococcus* and *Butyrivibrio*) diminished in the restricted animals, both lactic acid producer and utilizer bacterial groups increased(P<0.05). This showed a synchronization between *Streptococcus* and *Megasphaera,* suggesting that bacterial capacity to recover may be essential for avoiding metabolic disturbances onset.

PW319 Heavy metal contamination in soils, soil microbial community structure and nitrogen cycle - a case study using a mine slag from Zambia, sub-Saharan Africa

Yoshitaka Uchida[1], Miyuki Oka[2;3]

[1]Hokkaido University, Research Faculty of Agriculture, Sapporo, Japan
[2]Royal College of Art, School of Communication, Information Experience Design
[3], London, United Kingdom

Background: Heavy metal contamination of soil in the vicinity of mining sites is a serious environmental problem. The increasing levels of heavy metal contents in soils may negatively influence life supporting functions in soils, including nitrogen cycle process. Few studies investigated the effects of heavy metal contamination on soil microbial nitrogen cycle processes.

Objectives: To assess the quality of the soil in terms of the nitrogen cycle we focused on the dynamics of nitrate and ammonium, and bacterial community structure and functions within the soil.

Methods: We conducted an incubation experiment to investigate the effect of a slag containing high levels of Pb and Zn (62.2 and 33.6 g kg^{-1} slag as PbO and ZnO, respectively, sampled from a site formerly used as a lead and zinc mine) on the nitrogen cycle when mixed with soil (0–0.048 g slag g^{-1} soil). To assess nitrogen cycle, ^{15}N-labeled urea (500 mg N kg^{-1}) was added to the soil. Changes in soil pH, the concentration and ^{15}N ratio of nitrate (NO_3^--N) and ammonium, and bacterial relative abundance and community structure were measured.

Results: Results showed a slower NO_3^--N accumulation rates as the slag:soil ratio increased. Nitrification process was negatively influenced by the slag. *Firmicutes* had a positive and *Planctomycetes* a negative correlation with increasing slag concentration. Bacterial community functional analysis showed the proportion of bacterial DNA sequences related to nitrogen metabolism was depressed with increasing slag. We concluded that the slag impacted the soil bacterial community structure, and consequently influenced nitrogen dynamics.

PW320 protists are more sensitive to nitrogen fertilizations and show stronger seasonal dynamics than other microorganisms in three agricultural soil types

Zhi-Bo Zhao[1]

[1]*Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, State Key Laboratory of Urban and Regional Ecology, Beijing, China*

Background: Agricultural food production is at the base of human survival, and the application of fertilizers has fundamentally and continuously increased crop yield over the last decades. The productivity of crops is intimately tied to their microbiome, however, notoriously ignored in agricultural systems as fertilization disconnects the dependency of plants for often plant-beneficial microbial processes.

Objectives: We want to performed a holistic understanding of how soil microbiomes respond to the effects of fertilization regimes and season dynamics.

Methods: Here, we investigated the effect of a 2-year fertilization regimes (no nitrogen fertilization control, nitrogen fertilization, and nitrogen fertilization plus straw amendment) on entire soil microbiomes (bacteria, fungi, and protist) in three agricultural soil types cropped with maize in summer and autumn.

Results: The application of nitrogen fertilizers more strongly affected the taxonomic and functional composition of protist than bacterial and fungal communities. Nitrogen fertilizers significantly reduced the relative abundance of phagotrophic protists in the black and red soils in summer as a result of pH decreases. Generally, nitrogen fertilization indirectly reduced protist diversity. Nitrogen fertilizer plus straw amendment had greater effects on soil physicochemical properties and microbiome diversity than nitrogen addition alone. Moreover, nitrogen fertilization, even more together with straw, increased soil microbiome network complexity. Besides, protist community diversity and microbiomes networks also exhibit the obviously seasonal dynamics.

PW321 Genome-resolved metagenomics of oil-degrading microbial community in Arctic sea-ice

Jaak Truu[1], Kristjan Oopkaup[1], Marika Truu[1], Angela Peeb[1], Nga Phuong Dang[2]

[1]*University of Tartu, Institute of Molecular and Cell Biology, Tartu, Estonia*
[2]*Northern Research Institute Narvik, Narvik, Norway*

Background: There is evidence of oil biodegradation occurring at subzero temperatures but to what extent and which microorganisms are involved and how they contribute to the oil biodegradation process in the sea ice in the Arctic is still not well known

Objectives: The goals of this study were to assess oil-degrading microbial community structure and metabolic properties as well as to characterize metagenome-assembled genomes recovered from sea-ice mesocosms.

Methods: The experiment was performed in 120L mesocosms filled with the sea water. Crude oil was injected under the ice and ice with entrapped oil was kept at -14 °C for 3 months prior to melting for DNA isolation and subsequent shotgun metagenomic sequencing. Microbial community phylogenetic and functional diversity was assessed using a combination of different bioinformatics tools. Draft metagenome-assembled genomes (MAGs) were assembled from environmental DNA.

Results: Sea-ice with oil bacterial community was dominated by Pelagibacteriales, Rhodobacteriales and Altermonadales. Ten and three MAGs were recovered from control and oil-treated mesocosms, respectively. MAGs from oil treated mesocosms belonged to the genera Glaciecola and Ascidiaceihabitans. These MAGs possessed pathways for degradation of aromatic compounds, alkanes and synthesis of biosurfactants. Additional analysis revealed that two oil-degrading bacterial lineages, Roseobacter and Colwellia were abundant in obtained metagenomes but not recovered during the binning process.

PW322 Athalassohaline lagoon sediment bacterial community evolution in response to PAHs contamination

Sophie Marie Martirani Von Abercron[1], Patricia Marín[1], Silvia Marqués[1]

[1]*Estación Experimental del Zaidín, CSIC, Department of Environmental Protection, Granada, Spain*

Background: Polycyclic aromatic hydrocarbons (PAHs) are chemicals of particular environmental concern because of their stability, persistence in the environment and resistance to degradation. PAHs rapidly become associated with sediments, where they persist and bioaccumulate until degraded or resuspended. Sediments, as many natural habitats, are often anoxic. In these environments, the anaerobic degradation of aromatic compounds by microorganisms plays a major role in the removal of contaminants and nutrient recycling. In contrast to aerobic degradation of PAHs, much less is known about their anaerobic degradation.

Objectives: The aim of this work was to study the effect of PAHs on the bacterial communities in sediments and to enrich potential sulphate-reducing (SRB) naphthalene degraders.

Methods: We used sediments of the athalassohaline Fuente de Piedra lagoon to initiated enrichment cultures of SRB. The changes in the bacterial communities produced during enrichment were analyzed using Illumina MiSeq.

Results: *Proteobacteria*, *Bacteroidetes*, *Chloroflexi*, *Gemmatimonadetes* and *Firmicutes* were the most abundant groups in all the microcosms, as normally found in hypersaline environments. We observed significant changes during enrichment, which were strongly dependent on the medium used. Whilst synthetic salt medium designed for the selection of SRB produced a significant increase in the *Desulfobulbaceae* (*Deltaroteobacteria*), previously identified as aromatic degraders, in the cultures prepared with filtered water directly collected from the lagoon, *Firmicutes* was the most enriched taxon, where the genus *Dethiosulfatibacter*, a bacterial group probably related to PAH degradation, was dominant. We propose that natural media increase the recovery of the cultivable diversity of organisms of interest.

PW323 Insights into hydrocarbon degradation deploying anaerobic microcosms using Brazilian petroleum as sole energy source

Daiane Rodrigues Barbosa Belgini[1;2], Isabel Natalia Sierra García[1], Tiago Palladino Delforno[1], Jinwoo Kim[3], Lívia Soares Zaramela[3], Karsten Zengler[3;4;5], Valéria Maia Merzel[1]

[1]*Research Center for Chemistry, Biology and Agriculture (CPQBA), Microbial Resources Division, Paulinia, Brazil*
[2]*University of Campinas (Unicamp), Biology Institute, Campinas, Brazil*
[3]*University of California San Diego, Department of Pediatrics, La Jolla, United States*
[4]*University of California San Diego, Center for Microbiome Innovation, La Jolla, United States*
[5]*University of California, San Diego, Department of Bioengineering, La Jolla, United States*

Background: Petroleum is in high demands and a decline in its availability, especially high-quality oil, is becoming a reality. This is causing an economic impact for the oil industry and for global economy. Microbial metabolism can affect the petroleum quality through degradation of oil hydrocarbons. Thus, knowledge about the microbial impact on oil quality is crucial.

Objectives: The goal of this study was to evaluate the changes in biodegradation potential of microbial communities and the influence of different temperature and salt concentrations on hydrocarbon composition.

Methods: For this, methanogenic enrichments were prepared using oil as carbon source. After 35 weeks of incubation, subsamples were retrieved for shotgun metagenomic sequencing.

Results: Taxonomic profile of metagenomes showed *Petrotoga* as the most resilient genus to environmental changes. *Pseudothermotoga* genus was sensitive to condition changes, being overpassed by *Mesotoga* when the temperature decreased and was not detected when salinity increased. In all conditions, *Methanoculleus* genus was the most abundant archaeum. Functional analysis across all samples revealed that carboxylation was the main hydrocarbon activation process and few fumarate addition-related genes were detected. To complete the microbial community analysis, 34 high-quality metagenome assembled genomes were evaluated. Genomes affiliated to Deltaproteobacteria class presented genomic potential for toluene/benzene degradation through carboxylation activation. Those affiliated to Euryarchaeota phylum showed genomic potential for producing methane from methanol, acetate, and CO2/H2. In summary, these results show the main processes and taxa involved in hydrocarbon degradation as well as the impact that environmental conditions have on microbial composition and its metabolic potential.

PW324 L. pneumophila: biofilm production and the use of MALDI-TOF for its rapid discrimination

Teresa Maria Assunta Fasciana[1], Sara Cannella[1], Chiara Mascarella[1], Ignazio Arrigo[1], Miriam Sciortino[1], Maria Rita Tricoli[1], Salvatore Antonino Distefano[1], Domenico Graceffa[1], Anna Giammanco[1]

[1]*University of Palermo, Pro.Mi.Se, Palermo, Italy*

Background: *Legionella pneumophila* is a thin, aerobic, pleomorphic, flagellated, non-spore-forming, Gram-negative bacterium of the genus Legionella. *L. pneumophila* is the human pathogenic bacterium in this group and is the causative agent of Legionnaires' disease. For typing *L. pneumophila* different methods can be used, but in this study we considered pulsed-field gel electrophoresis (PFGE) and matrix-assisted laser desorption ionization timeof-flight mass spectrometry (MALDI-TOF-MS). In recent years, MALDI-TOF-MS has became a valuable for identification and typing of *L. pneumophila* isolates.

Objectives: We report the ability to produce biofilm and the use of MALDI-TOF-MS for typing in *L. pneumophila* strains.

Methods: Thirty-eight strains of *L. pneumophila* were isolated from environmental samples while two clinical strains were isolated from respiratory sapmles of patients admitted to different hospitals. All strains were analyzed by MALDI-TOF-MS, and were studied for biofilm productin according to G. Donelli et al.

Results: In our study we found that the 45% is a producer of biofilm, and it is divided in moderate maker strains (15%) and weak producer strains (30%). While clusterizzation by MALDI-TOF-MS we found that the strains belong to three different groups, particularly two clustereted together. Our results showed among the strains analysed a low biofilm producers suggesting the possibility to use the decontamination as the best strategies to prevent its colonization in water distribution systems.

PW325 Characterization of Roseobacter clade isolates with high capacities for aromatic compounds degradation

Antonio Bisbal[1], Balbina Nogales[1;2], Rafael Bosch[1;2]

[1]*Universidad de las Islas Baleares, Biologia, Area Microbiologia, Palma de Mallorca, Spain*
[2]*IMEDEA (UIB-CSIC), Esporles, Spain*

Background: Roseobacter lineage is a group of ubiquitous marine microorganisms abundant in coastal areas, marinas and harbours. Although there are evidences of their capability for degrading aromatic compounds and hydrocarbon degraders from this lineage have been isolated (i.e. *Salipiger aestuarii* 357, a naphthalene degrader isolated from crude oil- contaminated sand), none of them are as good degraders as naphthalene-degrading archetypes (i.e, *Pseudomonas stutzeri* AN10 and *Ralstonia eutropha* U2).

Objective: To isolate and characterize new strains of Roseobacter lineage with good capacities for aromatic hydrocarbon degradation.

Methods: Isolates were obtained in marine mineral medium with naphthalene from serial enrichments with aromatics hydrocarbons of different chronically-contaminated marine samples (water, sediment and tar balls). Isolates were analyzed by MALDI-TOF MS and those assigned to Roseobacter linage where further identified by 16S rRNA gene sequencing. Kinetic growth curves in naphthalene were performed. Eight best growing isolates were selected for genomic, metabolomic and physiologically analysis.

Results: The degradation pathway described for strain 357 was found in the new isolates with differences in gene order and in the presence of several transcriptional regulators, not found in strain 357. Other degradation pathways were also identified. All new isolates grew significantly faster than strain 357 in naphthalene. Our results confirm the existence of members in the Roseobacter lineage with hydrocarbon degradation capability similar to the present in the afore mentioned archetypes, that together with its great abundance in the marine habitat, suggest that Roseobacters might play an important role in the degradation of aromatic hydrocarbons in coastal areas.

PW326 Pantoea agglomerans colonization dynamics of the wheat roots upon germination

Ryszard Soluch[1], Nils Hülter[1], Tal Dagan[1]

[1]*University of Kiel, Institute of General Microbiology, Kiel, Germany*

Background: Plants are colonized by a wide diversity of microbes that may play a role in plant adaptation to various environmental conditions and disease resistance. Members of the plant microbiota include bacteria that are recruited from the environment and vertically inherited bacteria. The main road for vertically inherited bacteria in plants is considered to be transmission via the seed. Wheat seeds have been reported to harbor bacteria, however, the wheat embryo has been reported to be germ-free hence the colonization routs of seed-borne bacteria remain poorly understudied.

Objectives: Here we study the colonization dynamics of one member of the wheat microbiota – *Pantoea agglomerans* (*Pantoea*) – which we isolated from wheat seeds.

Methods: We established a system to cultivate germ-free plants devoid their native microbiota. In addition, we equipped the *Pantoea* isolate with broad-host-range plasmid encoding kanamycin resistance and constitutively expressing a fluorescent protein (GFP or mCherry). We followed the process of *Pantoea* colonization of the roots by determining the number of *Pantoea* cells per plant root system over time.

Results: Our results demonstrate dynamics of bacterial density increase. Five days post germination, *Pantoea* population reaches a stable carrying capacity of 10^8 cells/gr. Experiments of priority effects in colonization show that early incoming colonizers have a numerical priority over late incoming colonizers already after 24h. Our results suggest that seed-borne bacteria have an advantage in root colonization in wheat. This data provides a fundament for future experiments on association between *Pantoea agglomerans* and wheat host.

PW327 Syntrophy underlies co-occurence patterns between Christensenellaceae and Methanobrevibacter smithii in the human gut microbiome across populations

Albane Ruaud[1], Sofia Esquivel-Elizondo[1], Jacobo de la Cuesta-Zuluaga[1], Jillian Waters[1], Largus T. Angenent[2], Ruth E. Ley[1], Nicholas Youngblut[1]

[1]*Max Planck Institute for Developmental Biology, Microbiome Science, Tübingen, Germany*
[2]*Eberhard-Karls-Universität Tübingen, Tübingen, Germany*

Background: The dominant methanogen in the human gut microbiome, *Methanobrevibacter smithii*, positively associates with members of the bacterial family *Christensenellaceae* (order *Clostridiales*) across multiple human populations. *Christensenellaceae* are known to ferment dietary sugars to form CO2 and H2, substrates that *M. smithii* uses in methanogenesis.

Objectives: We investigated whether the co-occurence of these taxa is based on syntrophy.

Methods: Using mixed models, we verified the robustness of the association with an analysis of 1,821 metagenomes derived from 10 independent studies. Next, we tested for syntrophy with comparisons of mono- and co-cultures of *M. smithii* and *C. minuta*, a cultured representative of the *Christensenellaceae.* We measured gases and short chain fatty acids over time using gas chromatography and high performance liquid chromatography respectively, and visualized interactions with confocal and scanning electron microscopy.

Results: We confirmed a strong positive association between *Christensenellaceae* and *Methanobacteriaceae* families, and furthermore detected an association at the genus level between *Christensenella* and *Methanobrevibacter*. Culture experiments revealed that the bacterium efficiently supports the growth of the methanogen *via* copious H2 production. Moreover, *M. smithii* stimulated the production of acetate over butyrate by *C. minuta*, a change associated with increased H2 production. Thus, *M. smithii* shifts the metabolic output of *C. minuta*'s fermentation towards its own substrate. Finally, microscopy showed that *C. minuta* forms biofilms colonized by *M. smithii*, even when H2 is in excess. These results provide evidence of both metabolic and physical interactions, which likely underlie the co-occurrence patterns observed in the gut microbiome across populations.

PW328 Genomic and Transcriptomic Reconstruction of a Marine Flavobacterium Harboring Microbial Rhodopsins

Soon-Kyeong Kwon[1], Jihyun F. Kim[2]

[1]*Gyeongsang University, Divisionof Life Science, Jinju, Korea, Rep. of South*
[2]*Yonsei University*

Background: Microbial rhodopsins are commonly found among marine prokaryotes and function as light-driven ion pump to allow microbes to use light as an energy source and survive harsh environments. Our genome sequence analysis of the marine flavobacterium *Nonlabens* (*Donghaeana*) *dokdonensis* DSW-6 and other marine flavobacteria uncovered genes encoding an unexpected type of microbial rhodopsins dubbed NQ rhodopsins containing a unique motif. An NDQ rhodopsin was shown to function as a light-driven outward sodium pump (thus called NaR).

Objectives: To infer the physiological functions of microbial rhodopsins and the rhodopsin-related metabolic networks in *N. dokdonensis* DSW-6 that contains sodium- and proton-pumping rhodopsins.

Methods: The expression levels of the rhodopsin genes and others in the genome were examined during the growth under nutrient limitation and salinity stress in the light and the dark by RNA sequencing.

Results: Comparison of transcriptional information of *N. dokdonensis* DSW6 with light, nutrient and growth stage showed clustering results clearly distinguished according to growth stage and presence or absence of nutrients during cultivation. The expression level of proton-pumping rhodopsin was higher in the DSW6 strain at 2.5% salt concentration without nutrients. On the other hand, in case of sodium-pumping rhodopsin, the degree of expression was high at the 7.5% concentration of the nutrient-free salt. With the results of the rhodopsin-associated metabolic network analysis, it helps understanding the physiological features that allow survival in marine oligotrophic environments as a marine photoheterotroph, and estimating the ecological impact of microbial rhodpsins.

PW329 Colonization of phyllosphere communities at leaf emergence

Wenke Smets[1], Lucia Maria Spada[1;2], Isabella Gandolfi[2], Andrea Franzetti[2], Sarah Lebeer[1]

[1]*University of Antwerp, Department of Bioscience Engineering, Antwerp, Belgium*
[2]*University of Milano-Bicocca, Department of Earth and Environmental Sciences, Milano, Italy*

Background: Phyllosphere communities, the microbial communities at the plant-atmosphere interface, have a specific structure, depending on the host plant and the location. However, as trees shed their leaves every year, it is unclear how this consistent colonization happens.

Objectives: This study addressed how phyllosphere communities of emerging leaves are colonized. To do so, the relative importance of phyllosphere bacteria on leaf buds, branches, and trunk as bacterial sources was quantified. In addition, we studied what differences occur between communities of buds, branches, leaves, and trunk and we aimed to unravel the community dynamics of a colonizing leaf phyllosphere over time.

Methods: Eight London plane trees (*Plantanus x acerfolia*) in two different cities were sampled every week, throughout spring. At leaf emergence the sampling was more frequent. Community composition was studied using *16S rRNA* amplicon sequencing. Data were processed using Dada2 and analysed in R using a new package called "tidyamplicons".

Results: We found that 8% of the amplicon sequence variants (ASVs) in the leaf community came from the bud communities, 22% from the branches, and an additional 10% of the leaf ASVs came from the trunk. In addition, we found that community variation was most strongly determined by the sampled tree organ (buds, leaves, branch, or trunk). In addition community dynamics, expressed as beta dissimilarity with the previous time point, showed how long it takes for leaf communities to reach a relatively stable composition after leaf emergence.

PW330 Saccharomyces and candida sp as potential biosurfactant producers

Seun Adebajo[1], aderonke akintokun[2], Ridwan Abdulsalam[3], abidemi ojo[4]

[1]*Federal University of Agriculture, Microbiology, Abeokuta, Nigeria*
[2]*Federal University of Agriculture, Microbiology, Abeokuta, Nigeria*
[2]*Federal University of Agriculture, Microbiology, Abeokuta, Nigeria*
[4]*Federal University of Agriculture, Abeokuta, Nigeria*

Background: Biosurfactants are surface active compounds that reduce the interfacial tension between two liquids or between a liquid and a solid. Biosurfactants have different applications in accelerating bioremediation of hydrocarbon-contaminated sites, enhanced oil recovery, herbicides and pesticides formulations, detergents healthcare and cosmetics, pulp and paper, coal, textiles, ceramic processing and food industries etc. Biosurfactants are preferable over the chemical surfactants because of their effectiveness, degradability and environmentally friendly nature.

Objectives: This study focus on the isolation, identification and screening of biosurfactants by yeast isolates

Methods: In the present study, soil samples were obtained from three different oil contaminated sites at depths 0-10 cm and 10-20 cm respectively. Isolation of biosurfactant-producing yeast was carried out using yeast extract agar. Isolates were identified using morphological characteristics. Screening of biosurfactant producing strain was done using haemolytic assay, drop collapse test, microbial adhesion to hydrocarbon (MATH) assay and emulsification assay.

Results: Ninety-four yeasts were isolated. Haemolytic test showed that 25 isolates produced β haemolysis, 27 isolates produced α haemolysis and 42 isolates produced γ haemolysis. Twenty-two isolates out of the β haemolysis isolates were positive for drop collapse test. All the twenty-five isolates also showed various potential for adhesion to hydrocarbon test while 17 isolates had the ability for emulsification of hydrocarbon with the highest emulsification index of 61.1%. Yeasts isolated were mainly of *Saccharomyces* and *Candida* sp. Result obtained showed the potential of the yeasts isolates as good biosurfactant producers

PW331 Distribution of virulence genes in Vibrio species isolated from hospital wastewater effluents

Mashudu Mavhungu[1;2], Anthony Ifeanyi Okoh[2;3], Uchecwukwo Nwodo[2;3]

[1]*University of Fort Hare, Department of Biochemistry and Microbiology, , Alice, South Africa*
[2]*University of Fort Hare, SAMRC Microbial Water Quality Monitoring Centre, Alice, South Africa*
[3]*University of Fort Hare, Department of Biochemistry and Microbiology, Alice, South Africa*

Background: Vibrio species are emerging with significant clinical status; acquisition of new virulence traits. Hospital wastewater effluents, on the other hand, are globally alleged to serve as the puddle for the exchange of the virulence gene among the environmental bacteria owing to their capacity to encompass a vast diversity of clinical and environmental pathogenic bacteria.
Objectives: The distribution of virulence factor genes in some Vibrio species isolated from selected hospital wastewater effluents was evaluated.
Methods: Secondary hospital wastewater effluents (SWE), tertiary hospital wastewater effluents (TWE) and Limbede community wastewater effluents (LWE) samples were aseptically collected twice a week, over twelve weeks. Vibrio species identification and virulence factor genes determination were through the molecular method.
Results: About 270 isolates were confirmed as belonging to the Vibrio genus; SWE harboured 67% (182) as compared to TWE (14%) and LWE (19%). Upon speciation; 27% (73) was recorded as *V. cholerae*, 9% (25) as *V. parahaemolyticus*, 4% (12) as *V. vulnificus* and, 3% (8) as *V. fluvialis*. A relatively high distribution of virulence factor genes was shown; *ctx* (67%), *tcpA* (64%), *hylA* (81%), *ompU* (34%), *toxR* (88%), and *VPI* (58%) were present in *V. cholerae* isolated SWE and LWE. Also, *V. parahaemolyticus* harboured *Tdh* (56%) and *Tlh* (100%) while 100% of the *V. fluvialis* harbored *hupO* and *vfh* respectively. The abundance of virulence factor genes in the Vibrio species isolated from hospital wastewater effluent buttresses the need for pre-treatment of hospital wastewater effluents before discharge into a municipal wastewater treatment facility or the environment.

PW332 Ciliate protozoa affect the metabolic output and prokaryotic diversity of the rumen microbiome

Elie Jami[1], Bar Levy[1], Veronica Reiss[1]

[1]*Institute of animal science, Agricultural Research Organization (ARO), Volcani center, Department of ruminant science, Rishon LeZion, Israel*

Background: The rumen microbiome is composed of microorganisms spanning across all domains of life. Ciliate protozoa represent the most commonly found eukarya in the rumen comprising 50% of the microbial biomass. However, while the role of bacteria and archaea in the rumen has been the focus of many studies this past decade, the highly abundant eukaryotic components of the rumen microbiome remain underexplored and studies on the relationship between the protozoa population and the prokaryotic community in the rumen remain scarce.

Objectives: In this study, we assessed the effect of different rumen protozoa subpopulation on the metabolic output of the system and their surrounding prokaryotic community.

Methods: Rumen fluid was sampled in order to conduct an *in-vitro* semi-natural microcosm experiment in which the whole prokaryotic population was incubated with different protozoa subpopulations and with no protozoa for five days. Each day methane emission was measured and a subset of the sample was taken for prokaryotic composition analysis.

Results: We show that ciliate protozoa populations are associated with diverse and stable microbial populations that are significantly different from the free-living rumen microbial community. We further show that ciliate protozoa have a major impact on the metabolic output of the rumen microbiome that differs between the specific ciliate populations. We find that the presence of protozoa determines the amount of microbial fermentation products including methane and volatile fatty acids over time. In addition, we discover that the bacterial and archaeal diversity and abundance is highly altered by the protozoa presence over time.

PW333 Seasonal distribution of Vibrio spp. populations in the coastal waters of the Bay of Biscay

Maite Orruño[1;2], Elixabet Ogayar[1], Laura Alonso-Sáez[3], Vladimir R. Kaberdin[1;2;4], Inés Arana[1;2]

[1]*University of the Basque Country UPV/EHU, Department of Immunology, Microbiology and Parasitology, Leioa, Spain*
[2]*Research Centre for Experimental Marine Biology and Biotechnology (PIE-UPV/EHU), Plentzia, Spain*
[3]*AZTI Tecnalia, Marine Research Division, Sukarrieta, Spain*
[4]*IKERBASQUE, Basque Foundation for Science, Bilbao, Spain*

Background: Human activity profoundly changes the global environment by affecting not only the major biogeochemical cycles, but also the evolution of ecosystems. Thus, global warming and associated alterations trigger numerous changes in the physiology and distribution of organisms, including prokaryotes. Bacteria affiliated with the well-known genus *Vibrio* are widely distributed in aquatic systems, and have been suggested to be particularly responsive to climate change in marine systems. However, their presence and distribution in some coastal areas of great economic and recreational importance, such as the Basque Country, is unknown.

Objectives: To analyze the seasonal distribution and dynamics of *Vibrio* spp. populations in the coastal water of the Bay of Biscay.

Methods: During a one-year-period, coastal water samples were quaterly collected at 15 sampling stations from the Basque Monitoring Network. Samples were processed to determine the total bacteria (upon staining with acridine orange) and *Vibrio* spp. (by using CARD-FISH with the Vib572a probe). The number of culturable chemoorganotrophic bacteria and putative *Vibrio* spp. were determined by plating on Marine Agar and TCBS agar, respectively.

Results: Although the data were quite variable, in general counts of total bacteria and *Vibrio* spp. increased during summer and remained relatively constant during the rest of the year. In contrast, the percentage of culturable bacteria was lowest during the warmer season. Vibrios rarely exceeded 1% of total bacteria and their highest counts (occasionally above 10^4 cells/ml) were found in samples from the only station of transitional waters.

PW334 General Bacteriological Quality of Three Selected Rivers in Osun State, Nigeria

Atilade Adedeji[1], Ifeoluwa Omoboyowa[1], Mary Bisi-Johnson[1]

[1]*Obafemi Awolowo University, Department of Microbiology, Nigeria*

Background: Water has long served as a vehicle for the transmission of disease. Pathogens represent the most common etiological agent associated with waterborne illness. Contamination of water is a serious environmental problem as it affects the human health and the biodiversity adversely in aquatic ecosystems.

Objectives: This study was designed to determine the general bacteriological quality and prevalence of *Escherichia coli* O157:H7 in three selected rivers in Osun State

Methods: The rivers sampled were Esimirin in Ile-Ife, Asejire in Ikire and Erinle in Ede. Samples were analysed for Heterotophic plate count (HPC), Total Coliform Count (TC) and Feacal Coliform Count (FC) using spread plate methods on appropriate selective media. Biochemical properties of isolates were studied and further molecular confirmation were carried out on probable *Escherichia coli* O157: H7 isolates using Simple Polymarase Chain Reaction

Results: A total of 108 water samples were collected over a period of six (6) month consisting of both wet and dry seasons. Counts were highest during dry seasons for all the sampling sites and both seasons were beyond limits set by WHO and NCWR. HPC ranges from 2.85 ± 0.17 to 6.94±0.02 Log_{10}cfu/ml, TC was 2.15±0.07 -4.92±0.08 Log_{10}cfu/ml and FC was 1.49 ± 0.26 to 4.18±0.19 Log_{10}cfu/ml. *Vibrio* spp, *Aeromonas* spp. *Salmonella* spp*, Enterococcus* spp, *Pseudomonas* spp, *Bacillus* spp, *Escherichia coli* were recovered and E. coli O157: H7 was further confirmed from all sampling sites. Asejire was 11%, Erinle was 12% and Esimirin was 14%.

PW335 Initial Attachment for Different Ores by Leptospirillum ferriphilum, Acidithiobacillus ferrooxidans and Sulfobacillus thermosulfidooxidans

Javiera Norambuena[1], Michael Schlomann[1]

[1]*Leipziger Str. 29, Institut für Biowissenschaften, Freiberg, Germany*

Background: In bioleaching environments microorganisms are found in biofilms communities attached to minerals. The process by which these biofilms are formed is not completely understood. Bioleaching microbes can differentially attach to minerals; but not much information is available for initial attachment to these or other minerals.

Objectives: The objective of this project is to determine differential attachment to pyrite, chalcopyrite and sphalerite before 24 hours.

Methods: To accurately determine initial attachment to minerals we have established a qPCR procedure to determine cell numbers from organisms attached to mineral and free living. Exponentially grown cells (10^6 cells/ml) were treated with 2% of minerals (grain size 50-100 µm), samples are taken from the liquid phase (free-living cells) and from the mineral (attached cells) at different time points, frozen until DNA is extracted and cell numbers are extrapolated from a calibration curve with qPCR.

Results: In this study we have determined that *Acidithiobacillus ferrooxidans* and *Leptospirillum ferriphilum* quickly attach to pyrite and chalcopyrite, in less than 16 hours. On the other hand, *Sulfobacillus thermosulfidooxidans* was not attached at 24 hours, it required longer times. We focus the study on *L. ferriphilum*, because this organism has been less studied. Results will show attachment patterns of this microorganism with the three minerals and initial gene expression measured by qPCR of target genes. This study explores the initial attachment to minerals.

PW336 An insight into the microbial distribution and metabolic function of oligotrophic deep subsurface environment of Precambrian continental crust of Deccan traps of India

Dhiraj Paul[1], Swapnil Mundhe[2], Narendra Kadoo[2], Sukanta Roy[3], Yogesh Shouche[1]

[1]National Centre For Cell Science राष्ट्रीय कोशिका विज्ञान केंद्रा, Pune, India
[2]CSIR-National Chemical Laboratory, Pune, India
[3]CSIR - National Geophysical Research Institute, Hyderabad, India

Background: A very limited study has been done on microbial distribution and metabolic function of deep under the Earth's surface. Scientific exploratory boreholes at Koyna-Warna region of Deccan traps have provided a unique prospect to study the microbial structure and metabolic function at different depths of old basalts rock basement. The study site has great scientific interest due to frequent reservoir triggered seismicity (RTS) since the impoundment of the Shivaji Sagar Lake created by Koyna Dam in 1962.

Objectives: The present study aimed to investigate the extant microbial community structure, metabolic function of Koyna deep drilling site and understand its relationship with the geochemical environment.

Methods: Rock samples of different depth (500-850 m) was collected. Metagenome was extracted and amplicon library was prepared and sequenced using Illumina MiSeq platform. For global metabolomics, metabolites were extracted from the rock sample and analyzed using LC-MS analysis.

Results: Microbial diversity analysis indicated that bacterial phyla Firmicutes, Actinobacteria, Bacteroidetes, Thermi were detected predominantly in all the samples whereas Candidate_division_OPD, _AD3, and _OD1 were detected as a minor population. Presence of sulfur reduction/oxidation, nitrogen fixation/ reduction, and autotrophic organisms were noted in the deep biosphere samples. Global rock metabolomics analysis indicated the presence of an active community with sulfur, nitrogen and carbon metabolisms pathways. Therefore, the present omics study gives us an indication of how microbial life are survive and how metabolic pathways are involved in major biogeochemical cycle of deep basaltic region of Deccan Traps.

PW337 Spatial chaos: Can we predict patterns of spatial self-organization within microbial communities?

Felix Goldschmidt[1,2], Lea Caduff[1], David Johnson[1]

[1]*Eawag, Environmental Microbiology, Dübendorf, Switzerland*
[2]*ETH, Environmental Systems Science, Zürich, Switzerland*

Background: Spatially structured microbial communities comprise a vast amount of microbial life on our planet. They typically consist of different interacting species or strains that arrange themselves non-randomly across space (referred to as spatial self-organization). While spatial self-organization can have important effects on community-level properties, the underlying determinants of spatial self-organization remain unclear.

Objectives: We hypothesize that spatial chaos is one important determinant of spatial self-organization. More precisely, we propose that local differences in the initial spatial positioning of individuals can cause different patterns of spatial self-organization to emerge. We tested this hypothesis using a combination of experiments and mathematical simulations.

Methods: To test our hypothesis, we used a synthetic cross-feeding microbial community consisting of two isogenic strains of *Pseudomonas stutzeri*. One strain consumes nitrate to nitrite while the other consumes nitrite to nitrogen gas. We assembled the strains together and performed range expansion experiments on agar plates.

Results: Our experiments and mathematical simulations demonstrated that two fundamentally different patterns of spatial-self organization can emerge simultaneously as the microbial community expands across space. The simultaneous emergence of the patterns was not caused by spatial heterogeneity in the initial abiotic environment or by genetic heterogeneity within populations. Instead, it was caused by spatial chaos; local differences in the initial spatial positioning of individuals gave rise to the different patterns. We further demonstrated that the different patterns have different speeds at which they expand across space. Spatial chaos may therefore be an important determinant of the assembly and functioning of microbial communities.

PW338 Detection of Staphylococcus aureus in wild rabbits

Elena Moreno Grua[1], Sara Perez Fuentes[1], David Viana Martín[1], Laura Selva Martínez[1], Victor Lizana Martín[1], Jesús Cardells Peris[1], Juan Manuel Corpa Arenas[1]

[1]*CEU Cardinal Herrera University, PASAPTA, Alfara del Patriarca, Spain*

Background: *Staphylococcus aureus* is one of the most frequent pathogens in rabbit farms. However, there is few information about this bacteria in wild rabbits. These animals could be a source of infection for people and other animals, especially methicillin resistant strains (MRSA).

Objectives: The objective of this study were (1) to know the prevalence of *S. aureus*, (2) the most frequent genotypes and (3) the presence of methicillin resistance strains in wild rabbits.

Methods: For these purposes, 138 hunted animals from 5 different areas where an overcrowded of rabbits was detected in the Valencian Community were studied and swabs of the nostrils, ears and perineum were taken. These swabs were plated on blood agar plates, and the colonies compatible with *S. aureus* were grown in tryptone and soy broth. DNA was extracted and molecular typing of the coa gene was performed. To know the presence of MRSA isolates, PCRs of the *mecA* gene and the *mecC* gene were developed.

Results: We observed that 17.4% of the animals were positive to *S. aureus* and 8.7% of the animals were positive to the *mecC* gene. The types found in this study differed from previous studies on rabbit farms, indicating a possible adaptation of *S. aureus* strains to wild rabbits. These results highlight the need to continue studying this type of rabbits, in which the presence of MRSA strains has been already demonstrated, since they are in contact with other animal species and hunters.

PW339 Detection of viable cells of three pathogenic Legionella species by PMA-multiplex PCR

Jorge Garcia-Hernandez[1], Yolanda Moreno Trigos[2], Irene Hortelano Martin[3]

[1]*UPV, Centro Avanzado de Microbiologia de Alimentos, València, Spain*
[2]*UPV, Instituo de Ingenieria del Agua y del Medioambiente, València, Spain*
[3]*UPV, Instituto de Ingenieria del Agua y del Medioambiente, València, Spain*

Background: Although Legionella pneumophila is the most common specie detected, other species have been reported to cause human disease. The standard detection method by culture has a duration of at least 10 days. For detection of viable cells, a pretreatment with propidium monoazide followed by PCR has been developed by several authors.

Objectives: The purpose was to detect viable cells of four pathogenic species of Legionella using a PMA-multiplex PCR method.

Methods: Four reference strains (L. pneumophila , L. bozemanii , L. longbeachae and L. micdadeii) were studied. To determinate live and dead cells LIVE / DEAD system was used. The treatment with PMA at two different concentrations was tested for the 4 species in order to determine if it was effective to inhibit the amplification of non-viable cells. After treatment with PMA, samples were exposed to light phAST Blue. Four pair of specific primers were used and the multiplex PCR was carried out.

Results: It has been developed a method that combines a multiplex PCR with a PMA pretreatment that allows the detection of only viable cells of legionella spp. The proposed multiplex PCR method is not valid for the amplification of the 4 pathogenic species but allows the simultaneous detection of L. pneumophila, L. micdadei and L. longbeachae with a sensitivity of 10^3 cells.It is an specific and fast method (8 hours) that could be applied in environmental samples.

This study has been financed with grants for first projects (PAID-06-18) by The Polytechnic University of Valencia (UPV)

PW340 retrospective study of the first detection of florfenicol resistant gene in shiga toxin-producing escherichia coli isolated from slaughtered pork in korea

heo eunjeong[1], ko eunkyung[2], kim youngjo[1], park hyunjeong[1], wee sunghwan[2], Jinsan Moon[2]

[1]Ministry of Food and Drug Safety, Korea, Rep. of South
[2]Animal and Plant Quarantine Agency, Korea, Rep. of South

Background: Florfenicol is fluorinated derivative of chloramphenicol and represent highly protein inhibitors of bacterial protein biosynthesis. It has been commercially available in veterinary medicine or feed additives in the mid-1990s for pigs in Korea. The gene of the bacterial resistant to florfenicol from Salmonella Typhimurium DT104 and Escherichia coli (E.coli) should be identified in the worldwide.

Objectives: The antimicrobial resistance and resistance gene were investigated for Shiga toxin-producing E.coli isolated from pork in Korea.

Methods: We monitored 301 pork samples in slaughter houses and retail markets, and isolated 50 strains of E.coli form 2008 to 2009. Among these isolates, six isolates resulted in STEC. The minimum inhibitory concentration (MIC) on six strains was performed for 14 antibiotics, and three strains showed high MIC to florenicol and chloramphenicol (64㎍/㎖). PCR was conducted to detect the florfenicol resistant gene (floR) and the chloramphenicol resistant gene (cat).

Results: All of 3 strains contained the floR, while none of them had the cat. These PCR products were sequenced and aligned to obtain homology with other available gene in reference GenBank. A BLAST search showed that they contained sequences with homology to the floR gene of E.coli or Salmonella enteric serover Heidelberg. This is the first report to detect floR gene in STEC isolated from pigs in 2008. These results suggest that some STEC isolates in Korea carry florfenicol resistant gene and transfer this gene to other bacterial strains.

PW341 Detecting in a fast way the fish pathogenic viruses IPNV, IHNV, VHSV and VNNV

Andreia Silva[1;2], Mónica Nunes[1], Teresa Barreto Crespo[1;2]

[1]IBET - Institute of Experimental Biology and Technology, Food and Health, Oeiras, Portugal
[2]ITQB Nova, Oeiras, Portugal

Europe is one of the top consumers of fish with an annual average of 25.8kg/capita[1]. Fishing and fish consumption is a cultural and traditional habit with high impact in the all coastal country's economy. Ensuring that fish and fish products are safe for consumption is, therefore, of the upmost importance, being the development of rapid methods for detection and identification of diseases, an urgent need in the farming industry.

This study aims to identify and quantify four fish viruses in four important fish species from the Atlantic ocean, namely: the gilthead seabream and seabass, two of the most extensively farmed species in aquacultures and captured in the wild; and the European pilchard and the horse mackerel, two of the most consumed fish species in Portugal.

Two real-time multiplex PCR assays were developed for simultaneously detection of four species responsible for major economic losses in fisheries/aquaculture due to high morbidity/mortality: Infectious Pancreatic Necrosis Virus (IPNV), Viral Haemorrhagic Septicemia Virus (VHSV), Infectious Hematopoietic Necrosis Virus (IHNV), and Viral Nervous Necrosis Virus (VNNV). Mengovirus (vMC0) was used as control.

Real-time PCR assays as a singleplex and triplex for the detection of IPNV/IHNV/Mengo and VHSV/ VNNV/Mengo were successfully optimized with efficiencies between 90-110%, and a detection limit of 100 genome copies/µL. These qPCR protocols will next be applied to fish samples from Portuguese fisheries and aquacultures to better known the epidemiology of these viruses in the Atlantic coast. This methodology can then be used to control aquaculture production in fast and reliable way.

PW342 Efficacy of six different disinfection methods against artificial ESBL producting E. coli contamination on broiler hatching eggs and effects on hatchability

Gerzon Motola[1], Wiebke Tebrün[2], Michael Pees[2], Josef Bachmeier[3], Hafez Hafez Mohamed[1], Sarah Brüggemann-Schwarze[1]

[1]*Freie Universität Berlin, Institute of Poultry Diseases, Berlin, Germany*
[2]*University of Leipzig, Clinic for Birds and Reptiles, Leipzig, Germany*
[3]*BWE-Brüterei Weser-Ems GmbH & Co., Brüterei Süd ZN , Regenstauf, Germany*

Background: ESBL (extended-spectrum beta-lactamase) producing bacteria in poultry products are of major concern for the poultry industry and consumers. The present study focusses on the reduction of ESBL producing *E. coli* at the beginning of the broiler production chain to avoid a vertical transmission.

Objectives: This study focusses on testing the efficacy of six different disinfection methods against ESBL producing bacteria in broiler hatching eggs before incubation, and their effect on the hatchability.

Methods: The study is divided into two segments: Disinfection efficacy on eggshell samples and effect on hatchability. 20 sterile eggshells cutouts were used as carriers and artificially contaminated with 10^8 ESBL producing *E. coli* (strain 10682 CTX-M-1, phylogroup B1). Half of the samples were disinfected. Afterwards, cfu were determined for each and reisolation rates compared between disinfected and non-disinfected.

For the hatchability test, three groups were created with 50 broiler hatching eggs each; contaminated and not disinfected, contaminated and disinfected and neither contaminated nor disinfected. Contamination of the eggs was fulfilled using temperature difference egg-dipping in cold bacterial suspension. All groups were separated during the study. After 21 days of egg incubation, the hatching rate was determined.

Results: The six tested methods, namely Formalin fumigation, H_2O_2 + alcohol, essential oils spray and cold fog, peracetic acid and low energy electron beam, reduced the artificial contamination on the egg shell samples. Five of six methods had no negative effect on hatchability. Essential oils as spray reduced significantly the hatchability and will not be used for following field trials.

PW343 New insights into french cider microbial communities through metagenomics

Boris Misery[1], Cretenet Marina[1], Laplace Jean-Marie[1]

[1]*Caen, Caen, France*

Background: Calvados are distilled fermented apple juice drinks with a strong territorial impact due to their image and their local setting-up. Contrary to wine microbiology, only few studies deal with microbial community composition in fermented ciders and its incidence on Calvados organoleptic quality.

Objectives: Microbial diversity within ciders intended for distillation is explored in order to improve Calvados organoleptic quality. The microbial flora (yeast and lactic acid bacteria) is characterized at different steps of cider elaboration. Moreover, evolution of ciders from the same apple juice in two different fermentation conditions (directly from the tanks of the producer's cellar or in controlled conditions) are compared. Finally, microbial flora is correlated with the aromatic compounds quantifications found in cider.

Methods: Apple must samples were collected weekly from six producer's cellars throughout the Normandy region. DNA extraction was performed by NucleoSpin® Soil kit (Macherey). The microbial flora characterization was performed using NGS (Illumina MiSeq 2x 300 bp paired-end sequencing; V1-V3 16S region, ITS1 region). Aromatic compounds quantifications were performed by gas chromatography – flame ionization detector.

Results: The first metagenomics analysis ever conducted in fermented ciders is presented. Follow-ups of microbial populations show significant disparities in microbial flora according to the origin of the samples (producers and/or controlled conditions). Aromatic compounds profiles are statistically different between producer's cellars and are cross-analyzed with microbiological data to identify links between organoleptic characteristics and the microbial composition of cider. These results should enable us to define co-occurrences between bacterial flora and the aroma compounds found in Calvados.

PW344 Effect of controlled fermenting and distilling conditions on sugar cane molasses for the production of rum

Christian Coelho[1], Raphaelle Tourdot-Maréchal[1]

[1]*Institut Universitaire de la vigne et du vin - Univeristé de Bourgogne Franche-Comté, AgroSup Dijon, PAM UMR A 02.102, Dijon, France*

Background: Sugar cane molasses are the viscous end product of sugar companies that is mostly valued as raw material prior to fermentation and distillation for rum production purposes. The choice of yeast and the way fermentation is carried impact on the molasses wort chemical composition that will be revealed later in the characteristics of volatile composition of distillates.

Objectives: In this study, fresh fermented molasses were compared to yeast lees 3-months aged molasses in order to give rum producers the possibility to access to other rum styles and understand traditional fermentation protocols used in some countries for rum production. The way distillation is carried (coffey still or pot still) can still modify the volatile profile of final distillate and is also evaluated here.

Methods: Measuring molasses density and weigh losses and medium temperature monitored sugar can molasses fermentations. Microbiological controls are performed to attest the uniqueness of yeast in the fermentation processes. Distillates are finely analyzed by (i) targeting some rum volatile compounds of interest in the families of higher alcohols, esters, acetals and carbonyls (ii) discriminating fermentation/distillation processes by Excitation Emission Matrices of Fluorescence. Multivariate analyses made on chemical measurements enable to estimate the impact of how fermentation was carried on rum distillate profiles.

Results: Results from this experimentation highlight the chemical composition of rum distillates is totally dependent of the choice of combined fermentation and distillation processes that have to be implemented in the earliest stages of decisions to elaborate unique and controlled rum styles.

PW345 Lipid profile of oils from Irvingia gabonensis (Baill) seeds and its deterioration by a phytopathogenic fungal species

Adeniyi Sanyaolu[1], Adedotun Adekunle[2], Akinniyi Osuntoki[3]

[1]*University of Uyo, Botany and Ecological Studies, Uyo,*
[2]*University of Lagos, Botany, Lagos, Nigeria*
[3]*University of Lagos, Biochemistry, Lagos, Nigeria*

Background: As a survival strategy, fungi degrade most organic substances. In Nigeria, visually infected seeds of *Irvingia gabonensis* that are freely sold for human consumption in the open markets have been reported to contain diverse mycoflora. These phytopathogenic fungi are believed to be capable of affecting the lipids contained in these seeds.

Objectives: The work seeks to contribute to literature on the physico-chemical nature of oils from healthy and fungal infected seeds of *I. gabonensis* and to determine the corresponding nature of deterioration in the oil from *Aspergillus oryzae* infected seeds.

Methods: Using Soxhlet method, oil was extracted from healthy and *A.oryzae* infected seeds of *I. gabonensis*. Oil from both types of seed was subjected to a complete physico-chemical characterization. In addition, a Gas chromatographic profiling of the Fatty Acid Methyl Ester (FAME) was also done on both oils.

Results: Results from the study show that oil from *I. gabonensis* seed is composed of 12 fatty acids but lacking in caprylic, capric, lauric and margaric acids. The most abundant fatty acid present is the polyunsaturated Linoleic acid while the least abundant was the saturated myristic acid. Oil from *I. gabonenesis* seed is of a high nutritive and industrial quality. Also, infection of the seeds by *A. oryzae* significantly ($p = 0.05$) affected the physico- chemical attributes and fatty acid profile of the oil, thus confirming the ability of *A. oryzae* to deteriorate the quality and nutritional worth of oils from *I. gabonensis* infected seed.

PW346 Escherichia coli isolated from food as a risk factor to human health

Magda Janalíková[1], Silvie Pavlíčková[1], Darina Cejkova[2], Ivan Holko[3]

[1]*Tomas Bata University, Faculty of Technology, Department of Environmental Protection Engineering, Zlín, Czech Republic*
[2]*Veterinary Research Institute, Brno, Czech Republic*
[3]*Vetservis, s.r.o., Nitra, Slovakia*

Background: The *Escherichia coli* is considered heterogeneous species classified into four main phylogenetic groups A, B1, B2, and D which differ in genome sizes, virulence and antibiotic resistance profiles. To compete with other bacteria, many *E. coli* strains synthesize antimicrobial agents (colicins, microcins). The ecological role of bacteriocinogeny remains unclear. Whereas commensal strains help maintaining the balance in gastrointestinal tract, pathogenic strains can cause a broad range of human diseases including food poisoning.

Objectives: The aim of this study was to investigate the potential threat of *Escherichia coli* strains isolated from various food to human health.

Methods: *E. coli* strains originating from food (chicken, pork, and game meat, cheese, vegetables, cakes) were phenotypically examined for bacteriocin production and antibiotic resistance (EUCAST), followed by PCR detection of individual bacteriocin, antibiotic resistance, and virulence factor genes. In addition, whole genome sequencing (WGS) was also performed.

Results: Altogether, 160 *E. coli* strains were examined in this study. The most common resistances observed were against aminopenicillins; resistance to fluoroquinolones and aminoglycosides was also prevalent. Among isolated strains, 37% of bacteriocinogeny incidence (colicins: E7, E1, B, M, Ia; microcins: mV, mC7, mB17) was detected. ETEC and EIEC virulence factors were found in game meat and vegetables strains, on the other hand, APEC, UPEC and ExPEC virulence factors were proved in chicken meat and cheese strains. WGS generally confirmed the results detected by PCR. Thus, food can be a source of alimentary infection (game meat, vegetables) and also can be a vehicle of antibiotic resistance spreading.

PW347 Nondairy rice beverage produced by controlled fermentation with potential probiotic strain of Lactic acid bacteria

Hye Sun Choi*[1], In Duck Choi[1], Seuk Ki Lee[1], Ji Young Park[1], Nam Geol Kim[1], Sang Ik Han[1], Ha Cheol Hong[1]

[1]National Institute of Crop Science (NICS), Rural Development Administration (RDA), Suwon, 16613, Republic of Korea

Background: Milk and its fermentation products, such as yogurts, have been the probiotic carriers of choice for various reasons, including milk's recognition as a healthy product. However, more recent research efforts into probiotic potentials of cereal-based beverages are yielding remarkable results. There are a wide variety of traditional nondairy fermented beverages produced around the world.

Objectives: In this study, we developed a nondairy rice fermented beverage based on probiotic lactic acid bacteria (LAB).

Methods: We previously isolated probiotic *Lactobacillus* sp. from traditional Korean fermented soybean food, and rice fermented products were produced. The fermentation characteristics were analyzed and the properties of the metabolites were analyzed such as amino acid contents and antioxidant activities.

Results: The results showed that when *Lactobacillus* sp. were inoculated on the rice, the quality of fermented product were better than dairy product. And antioxidant effect and functional metabolite were increased. This study indicates that *Lactobacillus* sp. will enable as an appropriate starter for the development of functional nondairy rice fermented beverage. Our findings also could be used as therapeutic and functional supplement, to develop various foods.

PW348 Dynamics of microbial communities and metabolites during fermentation of ganjang, a Korean fermented soy sauce

Dong Min Han[1], Sang Eun Jeong[1], Byung Hee Chun[1], Che Ok Jeon[1]

[1]Chung-Ang University, Life Science, Seoul, Korea, Rep. of South

Background: Ganjang is Korean traditional fermented soybean sauce using raw materials including meju, fermented soybean lump, and solar salts. Because the fermentation process of ganjang is different depending on the manufactures' recipes, it is difficult to know the general fermentation features of ganjang.

Objectives: The aim of this study is to investigate the general fermentation features of ganjang using six different ganjang batches based on the analysis of microbial community and metabolite changes during entire ganjang fermentation periods.

Methods: Six ganjang batches were prepared from six different local ganjang manufacturers in South Korea according to the manufacturers' recipes and ganjang samples were collected during entire fermentation, periodically. The microbial community were analyzed based on the analysis of amplicon-based sequencing of V3-V4 regions of bacterial 16S rRNA genes and the fungal ITS2 region genes. Metabolites were analyzed using ^{1}H-NMR and PCA analysis were performed based on ^{1}H-NMR spectra.

Results: The bacterial community analysis at the genus level showed that the genera *Tetragenococcus*, *Staphylococcus*, *Chromohalobacter*, and *Bacillus* were identified from all ganjang batches although their relative abundances varied depending on ganjang batches. The genus *Debaryomyces*, known as a halophilic yeast, was identified from all ganjang batches as a dominant member and the genera *Aspergillus*, *Penicillium*, *Meyerozyma*, *Millerozyma*, *Wickerhamomyces*, and *Hyphopichia* were also identified as dominant members although their relative abundances were different depending on ganjang batches. The overall profiles of amino acids during ganjang fermentation were generally similar in all ganjang batches, but their concentrations were different depending on the ganjang batches.

PW349 assessing the efficacy of plasma activated water on mung bean seed safety and germination

Martina Darmanin[1], dmytro kozak[2], renald blundell[3], ruben gatt[2], vasilis valdramidis[1]

[1]University of Malta, Department of Food Sciences and Nutrition, Faculty of Health Sciences, Msida, Malta
[2]University of Malta, Metamaterials Unit, Faculty of Science, Msida, Malta
[3]University of Malta, Centre for Molecular Medicine, Biomedical Sciences Building, Msida, Malta

Background: Sprouts are prone to microbial contamination as foodborne pathogens within the seeds thrive in the favourable conditions of the sprouting process. The application of atmospheric pressure plasma is an innovative technique with the potential of reducing the biological contaminants on sprouts.

Objectives: This research aims to design a set-up for the surface activation of distilled water using plasma generated by direct current and atmospheric gas. The efficacy of the plasma activated water will be assessed in relation to its antimicrobial efficacy and seed sprouting.

Methods: Cathodic and anodic PAW samples were produced by surface treatment of water with plasma. pH, conductivity, and absorbance by optical emission spectroscopy (OES) was measured in all samples. The antimicrobial efficacy of the PAW was assessed against *Escherichia coli* NCTC 12900. The germination capacity (i.e., rate, %) of each PAW sample was tested on 40 mung bean seeds grown in test tubes containing 9 g glass beads and irrigated with 3 mL of PAW.

Results: The pH and conductivity of both PAW samples was lower than 3.3 and 122 µS/cm. OES spectral area indicated excitation in the range of 300 - 420 nm, with absorbance higher for the anodic sample. The antimicrobial and germination results indicate that while both PAW samples reduced the population of *E. coli* NCTC 12900 (> 4 log CFU/mL), no negative impact was observed in the germination rate of the seeds. A 10% reduction in the germination percentage was observed only in the case of the cathodic PAW.

PW350 Biocontrol of Staphylococcus aureus planktonic and biofilm cells using lytic bacteriophage

Minh Duc Hoang[1], Minh Son Hoang[1], Yoshimitsu Masuda[1], Ken-ichi Honjoh[1], Takahisa Miyamoto[1]

[1]*Kyushu University, Laboratory of Food Hygienic Chemistry, Department of Bioscience and Biotechnology, Faculty of Agriculture, Fukuoka, Japan*

Background: *Staphylococcus aureus* is a major cause of food poisoning due to its ability to produce enterotoxins and form biofilm. This opportunistic pathogen is also known as a serious public health threat because of the emergence of antibiotic resistant strains. Bacteriophages are increasingly considered as a promising alternative for the biocontrol of food borne pathogens.

Objectives: To isolate, characterize, and apply lytic phages to control planktonic and biofilm form of *Stapylococcus aureus*.

Methods: Bacteriophages were isolated using double layer agar technique. An isolated phage SA46-CTH2 was characterized by host range, morphology, one-step growth, and stability. The efficacy of SA46-CTH2 against planktonic cells of *S. aureus* SA46 was evaluated *in vitro* and in milk at various temperatures. The effect of phage SA46-CTH2 on biofilm of *S. aureus* SA46 was examined in microtiter plate and on stainless steel.

Results: A total of 29 phages were isolated from 54 food samples. Phage SA46-CTH2 was the most strongest lytic phage that could infect 95% of *S. aureus* strains tested. Morphology, one-step growth, and stability analysis indicated that SA46-CTH2 was a member of *Podoviridae* family, with relatively short latent period, large burst size, and high stability. Treatment with SA46-CTH2 significantly decreased planktonic cells of *S. aureus* SA46 in LB broth and milk compared to controls at all tested temperatures. Phage SA46-CTH2 also exhibited great efficacy in removing biofilm cells of *S. aureus* in microtiter plate and on stainless steel. These results suggested that phage SA46-CTH2 is a potential candidate for the biocontrol of *S. aureus*.

PW351 potential of gaba-producing bacteria isolated from fermented fish sauces as starters and probiotics

Vo Thi Thu Thao[1], Jun-Hyeok Yu[1], Jong-Hyun Park[1]

[1]Gachon University, Department of Food Science and Biotechnology, Seongnam-si, Korea, Rep. of South

Background: Gamma-aminobutyric acid (GABA) is a non-protein amino acid that have positive physiological activity. Enhancing GABA contents by using GABA-Producing Bacteria (GPB) is valuable job.

Objectives: The aim of this research was to isolate strains which have GABA-producing ability and qualify GPB as probiotics and starters for food application.

Methods: 91 strains were isolated from fish sauces, and evaluated their GABA producing ability by Thin-Layer Chromatography (TLC) and High Performance Liquid Chromatography (HPLC). Only 27 strains were confirmed their GABA-producing activity, and identified by 16S rDNA sequencing. Among 27 GPB, 5 strains belonged different genus (*Bacillus, Enterococcus, Lactococcus, Lactobacillus, Staphylococcus*) were evaluated as probiotics (NaCl resistance, 3% bile salt resistance, cell toxicity etc) and starters.

Results: Among 91 isolates, 27 strains had GABA-producing activity. Especially Lactococcus lactis LA43 isolated from Korean shrimp paste possessed high GABA-producing activity (454.18 mM) in MRS medium containing 500 mM monosodium glutamate. Selected five GPB had high stability at NaCl and low pH including antibacterial activity to pathogens, though were sensitive with 3% bile salt and successive digestion solution treatment. Nitrate oxide and cell proliferation assay revealed that heat-killed GPB enhanced activity of macrophage dose-dependently without any cytotoxicity. Furthermore, applied 5 GPBs could grow well at anchovy sauce.

PW352 Spatiotemporal variation of a Saccharomyces cerevisiae vineyard metapopulation in Santorini island

Ioanna Chalvantzi [1;2], Georgios Banilas[2], Pasxalis Theofanoudis [1;3], Aspasia Nisiotou[1]

[1]ELGO "DEMETER", Institute of Technology of Agricultural Products , Athens, Greece
[2]University of West Attica, Department of Wine, Vine and Beverage Sciences, Athens, Greece
[3]Agricultural University of Athens , Department of Food Science & Human Nutrition, Athens, Greece

Background: Accumulating data point to distinct grape microbial assemblages in different places, supporting the microbial terroir concept. However, there are still questions to be addressed, including the correlation of region-specific yeast populations with distinct enological characters and the temporal stability of regional genotypic signatures.

Objectives: The aim of this study was to estimate the genetic and phenotypic resemblances of geographically related *S. cerevisiae* vineyard populations and to detect their year-to-year genetic variation. To this end, vineyards of Santorini, a geographically isolated Aegean island of expected minimal external influence, was surveyed over three years.

Methods: *S. cerevisiae* strains isolated from 36 spontaneously fermented samples of 9 vineyards were genotyped through interdelta-PCR. The composition of vineyard populations showed spatiotemporal genetic variation. The total number of distinct genotypes per year ranged from 9 to 54. Interestingly, only 2 genotypes were repeatedly isolated over the three years, one of which dominated over time and space. The kinetic profiles and key enological parameters (pH, total and volatile acidity, organic acids, sugars, glycerol and ethanol) of different strains were determined and compared.

Results: Even within a small and relatively isolated region like the Santorini island, wine yeasts may exhibit biogeographic patterns. Considering all factors analyzed, the present study revealed significant year-to-year variation in the Santorini *S. cerevisiae* wine yeast metapopulation (PERMANOVA; F=8.3, p=0.0017).

«Acknowledgment: This research has been partially supported by the European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innovation, under the call RESEARCH – CREATE – INNOVATE (project code:T1EDK-04747) (MIS 5031228)»

PW353 Encapsulation of Lactobacillus casei in calcium alginate to use as probiotic in artisan icecream

Fabiola Cerda Leal[1], Jorge Albarrán Jeldres [2], Guillermo Petzold Maldonado[2], Rodrigo Nova[3]

[1]*University of Bio Bio, Chile*
[2]*University of Bio Bio, Chillan, Chile*
[3]*School of Veterinary Medicine and Science, U of Nottingham, United Kingdom*

Background: Adding probiotic microorganisms to food prodcuts could result in increasing their nutrinional value. Encapsulating microroganisms allow the survival of probiotic microorganisms making them available to the consumer.

Objectives: To evaluate survival of the probiotic bacterium Lactobacillus casei encapsulated in calcium alginate capsules when added to artisan icecream.

Methods: A presumptive Lactobacillus casei strain was isolated from a commercial food product and then identfied throug phenoticpic and molecular methods (Real Time PCR). Microcapsules of 2% calcium alginate were produced by extrusion. Vaibility of *L. casei* was assessd on R2A agar during storage at -20 degree Cecius during 33 days. Additionally, pH and drip loss of the product were measured.

Results: The extrusion method rsulted on firm and uniform size capsules, with an average size of 2.2mm. The encapsulated *L. casei* reached a concentration of 10^{14} cfu/g. The capsules allowed to recover viable *L. casei* post production and post starage at -20 degree Celcius, reaching a concentration of 10^{7} cfu/g post 15 days of storage, comapred with 10^{4} for non-encapsulated *L. casei*.There was no evidence of pH changes in the final product, with a lactic acid production of 0.2%. The product also showed a high water retention (Dripp loss 4.4), which was not linked to the presence of encapsulated bacteria.

PW354 Effects of supplementing a fishmeal diet with algae on the hindgut microbiome of farmed Atlantic cod (Gadus morhua)

Ciara Keating[1;2], James Hinch[3], Rob Davies[3], Alex Wan[4], Richard Fitzgerald[4], Umer Ijaz[1], Simon Davies[3;5], Majbritt Bolton-Warberg[6], Cindy Smith[1;2]

[1]*University of Glasgow, School of Engineering, United Kingdom*
[2]*National University of Ireland Galway, Microbiology, Galway, Ireland*
[3]*University of Plymouth, United Kingdom*
[4]*National University of Ireland Galway, Ryan Institute, Galway, Ireland*
[5]*Harper Adams University, Edgmond, United Kingdom*
[6]*National University of Ireland Galway, Zoology, Galway, Ireland*

Background: Efforts to farm Atlantic cod have been met with challenges (disease, early sexual maturation, high costs). Fishmeal (FM) feed represents 50% of the total cost in cod aquaculture. Initiatives have been undertaken to reduce costs of FM.

Objectives: We investigate supplementing a traditional FM diet with 5% seaweed (*Ascophyllum nodosum –'Asco'*) or 5% macroalgae (*Ulva rigida –'Ulva'*) and monitor the effect on fish health (via weight, hindgut SEM) and hindgut microbiome (16S rRNA amplicon sequencing) of Atlantic cod. The gut microbiome is intrinsically linked to health yet the impact of diet on fish gut microbiota is poorly understood.

Methods: Juvenile cod were graded into tanks and acclimated for 1 week while fed a control FM diet (time 0). Fish were then separated into one of three treatments; control diet; 'Asco' supplemented diet; 'Ulva' supplemented diet. Fish were sampled at time 0, week 8 and 12 whereby fish were euthanised and the hindgut removed for 16S rRNA amplicon sequencing analysis.

Results: Fish growth was significant lower in the 'Asco' treatment. Within this group varied growth led to fish being categorised as "Healthy" or "Unhealthy" (significantly reduced weight/length). Hindgut microvilli of 'Asco' fish were shown to be less dense. Microbiome data demonstrated no difference across treatments. Species richness was significantly greater at time 0. Photobacteria, Aliivibrio and Brevinema were dominant across all samples but decreased over time as Macellibacteroidetes, Fusobacterium and Bacteroidetes increased (except Asco-UnH). Thus in healthy fish, feed supplement did not change the microbiome, rather time was the driver.

PW355 Performance comparison of three different sampling strategies: FLOQSwabTM, rayon swab and sponges, for the detection of Listeria spp. and Listeria monocytogenes in a dairy plant

Erica Tirloni[1], Cristian Bernardi[1], Simone Stella[1]

[1]*University of Milan, department of health, animal science and food safety, Milan, Italy*

Background: The nylon FLOQSwab™ is a collection device well known for its performance in the clinical diagnostics, becoming object of studies in food industries as well.

Objectives: The first step of the study aimed to compare FLOQSwab™ and rayon tipped swab sampling in the detection of Listeria spp. and Listeria monocytogenes in a dairy plant producing PDO taleggio and PDO gorgonzola cheeses. The second step aimed to compare FLOQSwab™ with sponges.

Methods: In step one, samples were collected in three sessions: from 14 to 29 environmental points for each session were sampled with FLOQSwab™ and traditional rayon tipped swab. In step two, one sampling session was performed, considering 19 environmental points. All the samples were transported and submitted to analyses according to ISO 11290-1:2017.

Results: FLOQSwab™ resulted to be able to recover the microorganisms researched better if compared to the traditional rayon tipped swab: positive samples were 7.94% vs 1.59% for Listeria spp. and 4.76% vs 0% for L. monocytogenes with FLOQSwab™ and traditional rayon, respectively. The FLOQSwab™ showed also a better performance when compared to sponges (6 vs 1 positive samples for Listeria spp., respectively); no L. monocytogenes were detected. The FLOQSwab™, drastically improved the recovery efficiency of Listeria spp. and L. monocytogenes in the dairy plant, leading to suggest the use of this swab typology as a sampling tool for all the places difficult to reach in hygiene procedures of a dairy plant.

PW356 Development of a multiplex qPCR assay to detect common Pseudomonas species in raw milk

Christopher Maier[1], Jentsch Katharina[1], Lücking Genia[1], Wenning Mareike[1;2], Scherer Siegfried[1]

[1]Technical University of Munich, Chair of Microbial Ecology, ZIEL-Institute for Food & Health, Freising, Germany
[2]Bavarian Health and Food Safety Authority, Munich, Germany

Background: Spoilage of milk and milk products by bacterial peptidases can cause great financial loss for the milk industry. *Pseudomonas* is the most common genus in cold stored raw milk and some species are highly proteolytic. So far, there is only one heat-stable secreted peptidase known in *Pseudomonas*, the metallopeptidase AprA, which is encoded in the *aprA-lipA2* operon. Since the proteolytic activity varies strongly among different *Pseudomonas* species, a method that enables specific detection of most common and highly proteolytic *Pseudomonas* species is required for risk assessment of raw milk.

Objectives: Development of a multiplex qPCR assay in order to quantify frequently occurring, proteolytic *Pseudomonas* species and indirectly assess the risk of peptidase load in raw milk.

Methods: Whole genome sequencing of 56 raw milk isolates was performed by NGS. By means of six species-specific TaqMan probes and respective primers, binding to regions in the *aprA* gene a multiplex qPCR assay was designed, which detects nine *Pseudomonas* species commonly present in in raw milk.

Results: For all primer-probe pairs good PCR-efficiencies and high specificities were received in the multiplex qPCR approach. Moreover, significant correlations of cell counts from spiked raw milk with Ct-values measured by qPCR were obtained. For assay validation, 42 industrial raw milk samples were analyzed regarding their *Pseudomonas* content. All nine target species were detectable in different abundance, demonstrating the functionality and usefulness of this multiplex qPCR assay.

PW357 Comparative genomic and phenotypic analysis of Anoxybacillus flavithermus strains from dairy powder plants and raw milk

Anna Dettling[1], Christopher Huptas[1], Siegfried Scherer[1], Mareike Wenning[1;2]

[1]*Technical University Munich, ZIEL-Institute for Food and Health, Chair of Microbial Ecology, Freising, Germany*
[2]*Bavarian Health and Food Safety Authority, Food Hygiene, Oberschleißheim, Germany*

Background: The microbiota of milk powders is dominated by endospores of thermophilic bacilli, esp. of *Anoxybacillus flavithermus*. As they are found in raw milk only very rarely, their occurrence in powders must be due to alternative contamination routes during processing.

Objectives: Earlier studies identified strains of *A. flavithermus* that persisted in German powder production plants for several months. Hypothesizing that these strains were preferably selected and adapted to the processing environment, this study aims to understand the principles behind. A comparative analysis of 12 raw milk strains and 12 milk powder strains aims to discover properties that favor the development of persistence by being preferentially selected.

Methods: Phenotypic characteristics that are associated with persistence in production plants are biofilm formation, growth and sporulation behavior and the spore's resistance to cleaning procedures as applied in the plant. These were studied at laboratory scale using pure cultures of each strain. Additionally, the genome sequences were analyzed for occurrence of genes, gene-sets and functionalities.

Results: Spores of raw milk isolates of *A. flavithermus* differ significantly in their sensitivity towards processes involved in cleaning of the plant compared to powder isolates. Moreover, differences in fitness were detected as powder isolates showed better growth abilities and the demands on the environment were less strict.

PW358 Analysis of raw milk microbiome by amplicon sequencing - Impact of sample preparation and library PCR

Annemarie Siebert[1], Etienne Doll[1], Siegfried Scherer[1], Mareike Wenning[1;2]

[1]Technical University of Munich, ZIEL - Institute for Food & Health, Chair of Microbial Ecology, Freising, Germany
[2]Bavarian Health and Food Safety Authority, Oberschleißheim, Germany

Background: Since Next-Generation sequencing facilitates the detailed investigation of complex microbial communities, the analysis of raw milk microbiome gained increasing attention in recent years. However, raw milk is a highly complex matrix and a difficult object to analyze because in fresh raw milk a low density microbiota exacerbates the preparation of raw milk samples.

Objectives: Several DNA extraction methods are tested for yield of DNA after PCR-based library preparation for amplicon sequencing of raw milk. Furthermore, the introduction of artifacts by PCR and the resulting effect on diversity estimates is evaluated.

Methods: After raw milk pretreatment, bacterial DNA was extracted using DNA extraction kits based on enymatic lysis and mechanical lysis by bead-beating, respectively. Two-Step-PCR was used for PCR-based library preparation and amplicon sequencing was performed using an Illumina platform. Different cycle numbers in the first step of library PCR were applied to test for artifacts. Rhea-Pipeline was used for data analysis.

Results: Test of DNA extraction methods revealed that in addition to enzymatic lysis, mechanical lysis is inevitable for analysis of raw milk microbial communities to increase the yield of bacterial DNA used for library PCR. Also, cycle numbers in library PCR had a significant impact on the determination of the raw milk microbiome. However, replicate PCRs were reproducible. This study demonstrates the relevance of PCR conditions during library preparation, as those may influence the estimation of the abundance of particular bacterial groups as well as the biodiversity.

PW359 Presence of BlaTEM and CTX-M Genes in Enterobacteriaceae Isolates from Food Contact Surfaces at Selected Cafeterias within Bowen University, Iwo, Nigeria

Olufeyikemi Adeleke[1], Francis Jaryan[2], Abimbola Owoseni[3]

[1]*University, Biological sciences, Iwo, Nigeria*
[2]*National Public Health Institute of Liberia, Research Department, Monrovia, Liberia*
[3]*Bowen University, Department of Biological Sciences, Iwo, Nigeria*

Background: Enterobacteriaceae are common contaminants of food contact surfaces and potential distributors of antibiotic resistant genes. The growing threat from these resistant organisms call for concerted action to prevent the emergence of new and existing resistant strains.

Objectives: Experiments were carried out to identify antibiotic resistant genes in Enterobacteriaceae isolates from food contact surfaces at selected cafeterias in Bowen University, Iwo, Nigeria.

Methods: Members of the family Enterobacteriaceae were isolated by swabbing food contact surfaces in selected cafeterias using standard procedures. Bacterial isolates were identified through biochemical characterization and molecular test. The level of susceptibility of these isolates were then evaluated using antibiotic multidisc containing eight different antibiotics and resistance genes were amplified (using 16S RNA and DNA Extraction) from the resistant isolates.

Results: Ninety (90) enterobacteria isolates belonging to ten (10) genera were isolated and identified. *Enterobacter* and *Proteus* (27) had the highest occurrences followed by *Serratia* (11), *Klebsiella* (7), *Pantoea* (7), *Citrobacter* (4), *Escherichia* (2), *Morganella* (2), *Salmonella* (2), and *Hafnia* (1). 60% of the enterobacteria isolates were resistant to cefixime, 50% to cefuroxime, 36 % to ceftazidime and 1% to gentamicin while 70% were sensitive to nitrofurantoin, 66% to augmentin and complete susceptibility (100%) was observed to ciprofloxacin and ofloxacin. Nineteen of the isolates were resistant to two or more classes of antibiotics (multiple antibiotic resistance). Resistance genes were screened for in the antibiotic resistant isolates, sixteen showed the presence of Bla_{TEM}, and seven positive for Bla_{TEM} also showed the presence of *CTX-M* β-lactam resistant genes.

PW359 Probiotic properties of Lactic acid bacteria isolated from guava fruits

Tosin Olateru[1;1;2], Samuel Temitope Ogunbanwo [3]

[1]*Ibadan, Nigeria*
[2]*The Polytechnic, Ibadan, Biology, Ibadan, Nigeria*
[3]*University of Ibadan, Microbiology, Ibadan, Nigeria*

Background: Little information is available on the occurrence and pro biopic properties of lactic acid bacteria in guava fruits, hence the need for undertaking this study.

Objectives: To isolate and identity lactic acid bacteria from guava fruuits, to determine the probiotic properties of LAB isolated using various tests.

Methods: Enrichment culture method using MRS agar, morphological and biochemical tests, antagonistic activity of LAB isolates against indicator organisms, growth of LAB isolates at different concentration of bile salts and sodium chloride, and temperature tolerance of the isolates.

Results: All the LAB isolates are rod, the lactic acid bacteria isolates were able to inhibit the indicator organisms with different degree of inhibition; the isolated lactic acid bacteria were able to survive at different concentrations of bile salt, though the growth decreases with higher concentration of bile salt. Similar result was obtained for growth of the isolates with increase in temperature and higher concentration of sodium chloride. Statistical analysis revealed that there was no significant difference in the growth of the lactic acid bacteria with increase in the concentration of bile salt, sodium chloide and increase in temperature

PW360 The applicability of sourdough-related yeasts in the production of low FODMAP products

Vera Fraberger[1], Lisa-Maria Call[2], Konrad J. Domig[1], Stefano D'Amico[2]

[1]BOKU - Institute of Food Science, Department of Food Science and Technology, Vienna, Austria
[2]BOKU - Institute of Food Technology, Department of Food Science and Technology, Vienna, Austria

Background: FODMAPs (Fermentable Oligo-, Di-, Monosaccharides and Polyols), especially fructans, are assumed to play a major role in the pathological process of e.g. IBS (Irritable Bowel Syndrome). Wheat and wheat-based products are the most relevant sources of fructans in the Western European diet. However, a reduction of the FODMAP content in the diet of patients suffering from IBS was proven an effective approach to reduce symptoms. In this case, sourdough fermentation reveled to be a promising strategy to decrease the concentration of FODMAPs.

Objectives: The aim of this study was to investigate the potential of 13 sourdough-related yeast strains for their potential to decrease the FODMAP content.

Methods: The growth rate of the yeast strains was analyzed within a model system containing the typical carbohydrate profile found in wheat flour. In addition, for up to three days, these same samples were analyzed for changes within the carbohydrate composition by anion exchange-chromatography. Furthermore, a more realistic approach using wheat flour was performed to analyze the gas building capacity of each yeast strain, besides the reduction of total fructans, which was assessed by applying an enzymatic assay.

Results: Outcomes of this study revealed a highly different capability of the tested yeast strains to reduce carbohydrates, especially raffinose and fructans. However, the most significant changes within the model system and wheat flour suspension were observed for the sourdough isolates *Saccharomyces cerevisiae* and *Torulaspora delbrueckii*. In addition, these species exhibited superior CO_2 production, revealing their applicability for wheat bread production with improved leavening potential.

PW361 Zymomonas mobilis: an alternative dough leavener for the production of yeast-free baked doughs

Alida Musatti[1], Carola Cappa[1], Claudia Picozzi[1], Cristina Alamprese[1], Chiara Mapelli[1], Roberto Foschino[1], Manuela Rollini[1]

[1]*Università degli Studi di Milano, DeFENS, Milano, Italy*

Background: adverse responses to *Saccharomyces cerevisiae* occur in particular in people with Inflammatory Bowel Disease and with Crohn's disease, in which anti-*S. cerevisiae* antibodies directed against the phosphopeptidomannan of the yeast cell wall have been identified. *Zymomonas mobilis*, a Gram-negative bacterium GRAS classified by the FDA, can represent an interesting candidate for producing yeast-free fermented goods.

Objectives: as *Z. mobilis* does not utilize maltose present in flour, different strategies were applied to improve its leavening ability in dough: glucose or sucrose addition, or exploitation of the maltose hydrolytic activity of *Lactobacillus sanfranciscensis*.

Methods: five *Z. mobilis* and one *L. sanfranciscensis* strains were used in dough leavening trials. Experiments were set up with different dough consistency and inoculum (10^7-10^9 UFC/g), leavening temperature (25 - 30 ± 0.5 °C) and time (6 to 24 h), with or without sugars (3-5%) and NaCl (1%). Dough leavening properties (e.g., CO_2 production, dough volume increase, lag leavening time and rates) as well as time course of microbial population, sugars consumption and ethanol production were evaluated.

Results: the addition of glucose or sucrose as well as the highest leavening temperature increased the gaseous production and retention and consequently the dough development. *L. sanfranciscensis* contribution was fickle: the productivity gain showed positive performance only at high inoculum and short leavening time (3-4 h). NaCl worsened *Z. mobilis* fermentation performance but this behavior seems to be strain-related. Results highlight that developing a dough leavened by *Z. mobilis* and thus suitable for yeast-sensitive people is possible.

PW362 Dynamic follow-up of a Kombucha fermentation

Oceane Savary[1], Jérôme Mounier[1], Anne Thierry[2], Elisabeth Poirier[1], Marie-Bernadette Maillard[2], Camille Bourdier[1], Emmanuel Coton[1], Monika Coton[1]

[1]*Université de Bretagne Occidentale, LUBEM EA3882, Plouzané, France*
[2]*Institut National de la Recherche Agronomique, Rennes, France*

Background: Kombucha is an increasingly popular traditional beverage obtained by fermenting sweetened tea in the presence of a microbially rich floating biofilm. The final product contains organic acids and CO_2 and may contain low levels of ethanol along with other metabolites. During fermentation, a symbiotic relationship between yeasts and acetic acid bacteria and, in some cases, lactic acid bacteria has been described. Our previous work on industrial scale fermentations highlighted that *Acetobacter, Gluconobacter, Gluconacetobacter, Komagataeibacter* and *Oenococcus* bacterial genera and *Dekkera*, *Zygosaccharomyces* and *Hanseniaspora* yeast genera could be of technological importance for fermentations.

Objectives: The goal of this study was to dynamically follow a lab scale Kombucha fermentation using a selected microbial consortium.

Methods: Metagenetics (bacteria, yeast), microbiological, physico-chemical and biochemical (organic acids by LC-QTOF, volatiles by GC-MS) parameters were followed as well as biofilm formation using FISH-confocal microscopy and scanning electron microscopy over 27 days.

Results: Results showed sugar consumption by the microbial consortium and the successive production of multiple organic acids (especially acetic, lactic, gluconic and glucuronic acids) and volatile compounds, particularly volatile fatty acids, alcohols and their corresponding esters as well as a decrease in aldehydes over time. Active biofilm formation by acetic acid bacteria could also be monitored by microscopy and highlighted the distinct presence and positioning of the different microbial groups. These results provide novel information on the microbial dynamics of species within a defined consortium and their functional roles during fermentation and should lead to better fermentation control and product quality in the future.

PW363 Characterization of Shiga toxin-producing Escherichia coli isolated from flour from Swiss retails by whole genome sequencing.

Renate Boss[1], Jörg Hummerjohann[2]

[1]*Federal Food Safety and Veterinary Office, Bern, Switzerland*
[2]*Agroscope, Bern, Switzerland*

Background: Shiga toxin-producing E. coli (STEC) are serious foodborne pathogens. Typing STEC by the means of serogroups is decreasing while detection of virulence genes pattern achieves more and more relevance, including the use of whole genome sequencing (WGS).

Flour had only been recently considered as a vector for STEC transmission. It is a natural product and milling does not include a germ-reducing step. Flour is rarely eaten raw, but there is risk of consumption of unbaked dough.

Objectives: The aim of this study was to determine the frequency of STEC found in flour samples from the Swiss market and to fully characterize the isolates.

Methods: STEC were isolated from flour samples (n=93) after enrichment and charactrized by qPCR using assays according to ISO TS 13136. WGS was performed on a Illumina HiSeq platform and raw data were analysed by the pipeline of the European Reference Laboratory for STEC.

Results: We determined the frequency of STEC in the samples as 10.8% by PCR and gained 10 isolates with two samples being positive for two different STEC subtypes. Beside the isolation of one stx1- and stx2-positve STEC belonging to the “classical serogroups” frequently observed in outbreaks (O26), which could be a potentially cause for severe disease, we found several other common or less common STEC subtypes with diverse virulence patterns in flour. Our results are indicating both, the beneficial use of WGS as a characterization tool and flour as a potentially probably underestimated source for STEC infections in humans.

PW364 Impact of NDM-1 carbapenemase-producing Vibrio parahaemolyticus in imported seafood

Jens André Hammerl[1], Claudia Jäckel[1], Kathrin Behrmann[2], Silvia Schmoger[1], Nicole vom Ort[1], Eckhard Strauch[1]

[1]German Federal Institute for Risk Assessment, Department of Biological Safety, Berlin, Germany
[2]Landesuntersuchungsamt Bremen, Referat 20 – Mikrobiologie, Bremen, Germany

Background: Recently, a NDM-1-producing *Vibrio parahaemolyticus* recovered from an imported Vietnamese shrimp intended for human consumption in France was reported.
Vibrio parahaemolyticus is ubiquity present in aquatic environments, worldwide. The presence of this pathogen in food products pose a risk for humans, as the consumption of contaminated raw or undercooked seafood may cause gastrointestinal infections and/or septicaemia, especially in immunocompromised people.

Objectives: As the number of reports on carbapenemase-producing *Vibrio* spp. from imported fish and seafood of the Asian subcontinent increases, questions on the safety of imported food products from Asian countries arises. Thus, we investigated if antibiotic resistance surveillance should be extended to the environment close to human activities and foods of aquatic origin.

Methods: Antimicrobial resistance in was determined using the broth microdilution method according to CLSI guidelines and EUCAST epidemiological cut-off values. S1-PFGE, MiSeq-sequencing and bioinformatical analyses were performed to identify and characterize relevant isolates. The transferability of $bla_{\text{NDM-1}}$ carrying plasmids was investigated by *in vitro* filter-mating experiments.

Results: Antimicrobial resistance-testing among *Vibrio* spp. isolates from imported seafood, intended for consumption in Germany, exhibit a carbapenem-resistant *V. parahaemolyticus* isolate. MiSeq-sequencing revealed the presence of a $bla_{\text{NDM-1}}$ carbapenem gene that is embedded on a transposon, which is located on a self-transmissible plasmid. The $bla_{\text{NDM-1}}$ carrying plasmid could be transferred to *E. coli* resulting in a strong production of the carbapenemases. The plasmid was 100 kb in size and reflecting a mosaic structure. The composition and organization of the plasmid genome will be given in detail.

PW365 Impact of one Metschnikowia pulcherrima bio-protection strain in red winemaking

Raphaëlle Tourdot-Maréchal[1], Chloe Roullier-Gall[2], Scott Simonin[2], Hervé Alexandre[2], Jordi Ballester[2], Philippe Schmitt-Kopplin[3], Dominique Peyron[2], Stefania Vichi[4]

[1]Université de Bourgogne Franche-Comté , Institut Universitaire de la Vigne et du Vin, Dijon, France
[1]Université de Bourgogne Franche-Comté , Institut Universitaire de la Vigne et du Vin, Dijon, France
[3]Technische Universität München, Chair of Analytical Food Chemistry, Munich, Germany
[4]University of Barcelona, Dpt of Nutrition and Bromatology, Barcelona, Spain

Background: In a context of societal concern about food and wine preservation, along with the search for environmentally friendly productions, the reduction of sulphites is a major concern for the wine industry. The use of non-*Saccharomyces* yeasts as a bio-preservative has been recently proposed as a possible alternative to sulphites. Bio-protection consists in bacteria, yeasts or a mixture of microorganisms addition on grape must before fermentation in order to reduce the use of chemical compounds. A previous study in white winemaking showed that early addition of a non-*Saccharomyces T. delbrueckii* strain could be an alternative to sulphites (Simonin et al., 2018). However, there is a lack of scientific data concerning red winemaking.

Objectives: This study reports the analysis of a non-*Saccharomyces* yeast, *Metschnikowia pulcherrima*, inoculated at the beginning of the red winemaking process in three wineries as an alternative to sulphiting.

Methods: Fermentative kinetics, polyphenols composition, volatiles compounds, sensory and metabolomics profiles have been recorded.

Results: The implantation of the *M. pulcherrima* was successful in all the wineries and effectively limited the development of spoilage microorganisms in the same way as the addition of sulfites. The study revealed that the addition of *Metschnikowia pulcherrima* could protect must and wine from oxidation and could replace sulphites. Moreover, the metabolomics analysis using ultra high resolution FT-ICR-MS highlighted a specific bio-protection signature.

PW366 Multiple molecular components of Listeria monocytogenes affected by high pressure processing treatments: Fourier transform-infrared spectroscopy insights

Teresa Goncalves Ribeiro[1], Florentina Bucur[2], Daniela Borda[2], Carla Novais[1], Patrícia Antunes[1;3], Anca Nicolau[2], Nadav Bar[4], Luísa Peixe[1]

[1]UCIBIO/REQUIMTE. Faculty of Pharmacy, University of Porto, Porto, Portugal
[2]Dunarea de Jos University of Galati, Galati, Romania
[3]Faculty of Nutrition and Food Sciences, Porto, Portugal
[4]Norwegian University of Science and Techology, Norway

Background: High pressure processing (HPP) is an attractive alternative technology to conventional thermal treatments for inactivation of foodborne pathogenic bacteria. Despite its interest, the effect of HPP on bacterial cellular components is not well established, undermining the development of strategies for circumventing the emergence of HPP-tolerant bacteria.

Objectives: To evaluate the impact of different HPP treatments on molecular components of *Listeria monocytogenes.*

Methods: Fourier-transform infrared with attenuated total reflectance (FTIR-ATR) spectra of two clinically-relevant *L. monocytogenes* strains (RO15-serotype 4b/herring+spices/Romania/2013; ScottA-1/2a/milk/France/1992) were acquired from stationary phase growth suspension cells, exposed to HPP treatments (300MPa-2/8/15'; 400MPa-2/8/15'; 600MPa-15') or not, using Nicolet iS50 FT-IR spectrometer (6 replicates/resolution of 4cm^{-1}/32 scan co-additions), and modeled with hierarchical cluster analysis (HCA) and partial least squares discriminant analysis (PLSDA).

Results: Strain specific spectra were observed before and after HPP treatments by HCA. *L. monocytogenes* cells submitted to HPP were clearly discriminated from non-treated cells by PLSDA, with variances occurring in all spectra. Three clusters were evidenced for each strain by HCA, corresponding to cells exposed to 300MPa-2', 400MPa-2' or 600MPa-15'. Additionally, all but two (RO15: 300MPa-8/15', ScottA: 400MPa-8/15') HPP treatments were discriminated by PLSDA, with multiple cell components being affected. Nevertheless, the main spectral variances were observed in proteins/amides I and II (1700-1500cm^{-1}) and in phospholipids/DNA/RNA (1500-1200cm^{-1}) regions, which seems to be the main targets of HPP. Elucidations of these cellular components are crucial for enhancing HPP efficacy (e.g. development of specific HPP additives) and for studying bacterial HPP tolerance responses.

PW367 Metagenomic approach reveals microbial diversity and predictive microbial metabolic pathways in hongeo, a traditional korea fermented skate product

ChangCheng Zhao[1], SuJung Hwang[2], Jong-Bang Eun[1]

[1]Chonnam National University, Food Science and Technology, Gwangju, Korea, Rep. of South
[2]Daegu Haany University, Faculty of Herbal Food Cusine and Nutrition, Daegu, Korea, Rep. of South

Background: *Hongeo* is a typical traditional fermented fish product in Korea, and it is made up of fresh skate without any additives in a ceramic jar over 1 weeks at room temperature.

Objectives: In the present study, metagenomic approach and culture dependent technology were applied to describe the diversity of microbiota and identify beneficial microbes in *hongeo* samples.

Methods: DNA libraries were constructed after extracting bacterial DNA from fermented skate. A whole-genome shotgun sequencing was performed by using IIIumina HiSeq2000. The bacterial diversity and gene prediction were studied.

Results: At the family level, *Pseudomonadaceae*, *Moraxellaceae*, *Carnobacteriaceae* and *Clostridiaceae* were the major abundant families in five fermented skate samples. PCoA based on Weighted UniFrac distances showed an apparent clustering pattern for hongeo samples from different locations, and *Betaproteobacteria*, *Fusobacteria*, *Oceanospirillales*, *Alteromonadales* and *Lactobacillales* represented OTUs (operational taxonomic unit) according to the major identified markers. At the microbial functional level, it was observed that most genes belong to function unknown group, followed by replication, recombination and repair groups and cell wall/membrane/envelope biogenesis groups. There was an enrichment of metabolic functional features, including carbohydrate and amino acid transport and metabolism, and inorganic ion transport and metabolism, which implied that the microbial metabolism in the skate samples tended to be vigorous. The diverse microbial composition and high metabolic vigor of the microbes present in fermented skate samples may render them suitable for further exploration and appropriate applications by the scientific community.

PW368 Horizontal transfer of plasmid encoding lincomycin resistance gene during soybean fermentation and the passage of intestine in mice

Do Won Jeong[1], Sojeong Heo[1], Taeok Bae[2], Jong-Hoon Lee[3]

[1] Dongduk Women's University, Department of Food and Nutrition, Seoul, Korea, Rep. of South
[2]Indiana University, Department of Microbiology and Immunology, Gary, United States
[3]Kyonggi University, Department of Food Science and Biotechnology, Suwon, Korea, Rep. of South

Background: The pSELNU1 contains a lincosamide nucleotidyltransferase (*lnuA*) gene which confers lincomycin resistance. Previously, we showed that the pSELNU1 plasmid is transferred to other bacteria in laboratory growth conditions. However, it is not known whether the plasmid can be transferred to other bacteria during food fermentation (*in situ*) or during passage through animal intestine (*in vivo*).

Objectives: We examine the in situ and in vivo transfer of pSELNU1 using *Staphylococcus saprophyticus* as a recipient.

Methods: To check the in situ transfer during fermentation, *S. equorum* containing pSELNU1 as a donor strain and *S. saprophyticus* as a recipient were inoculated into soybean. To check the in vivo transfer during the passage of intestine in mice, both of donor and recipient strains was administered into mice via oral gavage.

Results: During soybean fermentation, the plasmid was transferred at a rate of $1.9 \times 10^{-5} – 5.6 \times 10^{-6}$ per recipient. During passage through murine intestine, the plasmid showed similar transfer rates from 2.3×10^{-5} to 7.1×10^{-6}. Notably, the transconjugants were found only in the presence of the antibiotic, confirming that, like the transfer of antibiotic resistance genes in hospitals, the antibiotic selection is the key for the resistance strains. Our results show that, in the presence of corresponding antibiotics, the food associated *S. equorum* can serve as a donor of antibiotic resistance plasmid and spread the antibiotic resistance.

PW369 Starter culture fermentation of banana (Musa sapieutum) juice with indigenous yeast strains for Agadagidi (Nigerian alcoholic beverage) production

Bridget Omafuvbe[1], Stella Adeyemo[1], Olaide Balogun[1]

[1]*Obafemi Awolowo University, Department of Microbiology, Ile-lfe, Nigeria*

Background: Ripe banana (*Musa sapientum*) fruits perish in tonnes during harvesting season , posing environmental problems in Nigeria. To prevent waste, overripe banana can be processed into *Agadagidi* – a traditional not well known fermented alcoholic beverage. *Agadagidi* is traditionally produced on small scale, house hold basis under variable environmental conditions, resulting in unpredictable quality.

Objectives: To select suitable indigenous yeast strain(s) from traditionally produced *Agadagidi* for starter culture fermentation of the product with improved quality and safety.

Methods: Ripe banana fruits were peeled, crushed in distilled water (1:3 w/v), filtered through muslin cloth and filtrate pasteurised. *Saccharomyces* species (*S. cerevisiae* , *S. bayanus* and *S. kudriavzevii*) isolated from traditionally produced *Agadagidi* were used (singly and in combinations) to ferment the banana juice at 30°C for 72 h into *Agadagidi*. Titratable acidity (TTA), °brix, pH, specific gravity (SG) and alcohol content were monitored during the fermentation process following standard methods. Sensory evaluation was carried out on both natural and starter culture produced *Agadagidi*

Results: The pH, °brix and SG decreased from 4.8 to 4.1; 5.4° to 1.7° and 1.021 to 0.998 respectively, while TTA and alcohol content increased from 0.13% to 0.38% (lactic acid) and 0.0 to 2.93% (ethanol) in the fermentations. Organoleotically, agadagidi produced by *S. cerevisiae* as single starter was most acceptable and significantly different from the natural fermented product. The result obtained is useful in improving the quality and possible scale up of the production, to utilise the readily available substrate, prevent waste and environmental problems.

PW370 Identification and antibiotic resistance of enterococci isolated from fresh fruits and vegetables

Nejib Guizani[1], Zahra Al Kharousi[2], Abdullah M. Al Sadi[3], Ismail Al Bulushi[2]

[1]*Sultan Qaboos University, Food Science and Nutriton, Al Khod, Oman*
[2]*Sultan Qaboos University, Food Science and Nutrition, Al Khod, Oman*
[3]*Sultan Qaboos University, Crop Science, Al Khod, Oman*

Background: Fruits and vegetables can be inhabited by pathogenic bacteria and/or antibiotic resistant bacteria. *Enterococcus* bacteria are able to survive adverse environmental conditions and have been involved in nosocomial infections, food intoxication and in spreading antibiotic resistance through the food chain.

Objectives: To identify enterococci in 13 types of fresh fruits and vegetables, screen them for their resistance to antibiotics and identify some antibiotic resistance genes.

Methods: Enterococci were isolated from 13 types of local and imported fresh produce on Slanetz Agar (SA). The bacteria were identified by PCR targeting bacterial 16S rRNA gene and screened for their resistance to antibiotics by disc diffusion method. Tetracycline-resistance genes were identified using multiplex PCR.

Results: Eight species of Enterococcus (31 isolates) were identified. The most common specie were *Enterococcus casseliflavus*, *Enterococcus faecalis*, and *Enterococcus mundtii*. Two isolates of *Enterococcus sulfureus* and *Enterococcus faecium* and one isolate of *Enterococcus gilvus*, *Enterococcus hirae*, and *Enterococcus raffinosus* were also identified. *Enterococcus* bacteria showed resistance to chloramphenicol and tetracycline, resistance/intermediate resistance to erythromycin and intermediate resistance to ciprofloxacin and vancomycin. Sixteen isolates of *Enterococcus* showed multiple antibiotic resistance against 2 or more antibiotics from different classes. The *tet* genes: *tet*(K), *tet*(L) and *tet*(M) were detected in *Enterococcus sulfureus*, *E. mundtii*, 2 isolates of *E. casseliflavus* and 2 isolates of *E. faecalis*. These results indicate that the group of *Enterococcus* may influence the resistome of fresh produce and that fresh fruits and vegetables may aid in dissemination of enterococci or their antibiotic-resistant genes to different environmental niches.

PW371 Proteomes of cells and spores of an IPTG-inducible kinA strain of Bacillus subtilis versus wild-type

Zhiwei Tu[1;2], Wishwas Abhyankar[1;2], Bhagyashree Swarge[1;2], Chris de Koster[2], Stanley Brul[1], Leo de Koning[2]

[1]Swammerdam Institute for Life Sciences, University of Amsterdam, Molecular Biology and Microbial Food Safety, Amsterdam, Netherlands
[2]Swammerdam Institute for Life Sciences, University of Amsterdam, Mass Spectrometry of Biomacromolecules, Amsterdam, Netherlands

Background: *Bacillus subtilis* can form dormant spores able to survive harsh conditions. A phosphorelay controls the initiation of sporulation, starting with auto-phosphorylation of the kinase KinA. KinA transfers phosphoryl groups to Spo0A, through Spo0F and Spo0B. Accumulation of Spo0A~P_i above a threshold triggers sporulation.

Objectives: To perform time resolved proteomics on sporulating cells synchronization of sporulation is needed. To that end we induce *kinA* expression in *Bacillus subtilis* bearing *kinA* under control of an IPTG responsive *hyperspank* promoter ($P_{hy\text{-}spank}$).

Methods: $P_{hy\text{-}spank}$ *kinA* was grown in a defined, MOPS-buffered medium with 40 mM glucose. 100 µM IPTG was added when the culture reached OD_{600} 0.7 and growth was continued for 90 minutes. Next, the culture was diluted with medium devoid of glucose. Cells and spores were harvested at the moment of glucose dilution and 1 day after that. Cells and spores of *B. subtilis* PY79 were harvested similarly. Minimal medium was used to metabolically label proteins with ^{15}N by replacing $^{14}NH_4Cl$ with $^{15}NH_4Cl$. Electrospray Ionization (ESI)-FTICR Mass spectrometry was used to quantitatively analyze tryptic protein digests. Wet heat resistance and germination behaviour of spores were tested according to established protocols.

Results: IPTG induced and not induced spores and spores of the wild-type strain showed equivalent resistance to wet heat but were delayed in phase-darkening (germination *sensu strictu*). Comparing $P_{hy\text{-}spank}$ *kinA* and wild-type cells showed that a group of sigma A controlled genes and 16 out of 20 germination related genes were downregulated. The latter could explain the observed delayed spore germination.

PW372 Origin of spore-forming spoilage organisms in dairy products

Etienne Doll[1], Anna Dettling[1], Scherer Siegfried[1], Mareike Wenning[2]

[1]*Technical University Munich, Chair of Microbial Ecology, Freising, Germany*
[2]*Bavarian Health and Food Safety Authority, Oberschleißheim, Germany*

Background: Spore-forming bacteria in food can occure as spoilage organisms or may even constitute a health risk. However, many dairy products like extended shelf life (ESL) milk or milk powder are manufactured at temperatures that do not inactivate heat-resistant spores or, in case of thermophilic spore-formers, even select for their growth.

Objectives: Spores may be introduced into the process via raw materials, but may also originate from recontamination events. To develop strategies against the entry of spore-formers in dairy products the project focussed on the detection of contamination routes.

Methods: To investigate the correlation of spores in raw material and end products we compared their counts and biodiversity in bulk tank milk, ESL milk, and milk powders. Additionally, strains from different process stages were sub-typed to substantiate transmission, long-term persistance and recontamination events within the plant.

Results: The raw material had only a minor impact. Spore-counts in bulk tank milk were low and especially during the production of ESL milk they were further decreased by almost 4 log-units. Additionally, species like *Bacillus cereus,* responsible for premature spoilage of ESL milk, or *Anoxybacillus flavithermus* that often reached high counts in milk powder, were rarely isolated from raw materials. Instead, identical strains of both species occurred in end products of multiple batches that were produced within several weeks or even months. Those strains likely survived the cleaning procedures, persisted within the plant and recontaminated the product. Thus, an optimized plant sanitation is a key parameter for limiting counts of spore-formers in dairy products.

PW373 Cellulose Nano Fibers (CNF) - Sakacin-A active package to reduce Listeria population in smoked salmon

Chiara Mapelli[1], Alida Musatti[1], Alberto Barbiroli[1], Paolo Motta[1], Seema Saini[2], Manuela Rollini[1]

[1]Università degli Studi di Milano, DeFENS, Milano,
[2]LGP2 Laboratoire Génie des Procédés Papetiers, Grenoble INP,

Background: Sakacin-A is a class IIa bacteriocin produced by the LAB *Lactobacillus sakei*; like all bacteriocins belonging to this class, it exerts an antimicrobial effect against the causative microorganism of listeriosis, a highly fatal opportunistic foodborne infection. An interesting feature of this bacteriocin is its potential to be delivered through active packaging materials for food which could support *Listeria monocytogenes* growth.

Objectives: High yield production of a protein extract enriched in sakacin-A, employing a low-cost medium formulated with cheese whey permeate; the extract will be used to produce active films, whose antimicrobial activity will be tested *in vitro* and *in vivo* trials.

Methods: *L. sakei* DSMZ 6333 was grown in a 14 L fermenter. Sakacin-A was purified from the cell-free supernatant by ammonium sulphate precipitation and lyophilization. The produced sakacin-A extract was adsorbed onto cellulose nanofibers (CNF) to obtain active films that were characterized by infrared spectra and thermo-gravimetric analyses. Antimicrobial trials were carried out *in vitro* using *Listeria innocua* as an indicator strain; results were also confirmed *in vivo,* employing intentionally inoculated smoked salmon (10^3 cells/g).

Results: 4.51 g of freeze dried protein extract were obtained from 1 L supernatant, containing about 75400 AU. A clear halo of inhibition was observed in *in vitro* tests around *L. innocua* solid cultures; *in vivo* tests yielded a similar outcome*:* in smoked salmon, *L. innocua* population was reduced by about 2.5-3 Log cycles after 28 days at 6°C in presence of the active films, compared with negative control without the bacteriocin extract.

PW374 Staphylococcus aureus related to bovine mastitis in Switzerland: clonal diversity, virulence gene profiles and antimicrobial resistance of isolates collected throughout 2017

Nadine Käppeli[1], Marina Morach[1], Sabrina Corti[1], Corinne Eicher[1], Roger Stephan[1], Sophia Johler[1]

[1]*University of Zurich, Institute for Food Safety and Hygiene*

Background: *Staphylococcus aureus* causes bovine mastitis, leading to considerable financial losses to the dairy industry. In addition, milk products are one of the most common food categories implicated in Staphylococcal Food Poisoning.

Objectives: Our objective was to assess the population structure, phenotypic resistance patterns and virulence and resistance gene profiles of *S. aureus* isolates from bovine mastitis milk in Switzerland.

Methods: A total of 58 strains were characterized by DNA microarray, *spa* typing, MLST, and determination of minimum inhibitory concentrations of antimicrobial agents.

Results: The strains were assigned to seven clonal complexes, 10 sequence types and 11 *spa* types, with CC705 (43%), CC97 (33%), and CC20 (12%) representing the most common lineages and t529 (43%) and t267 (21%) representing the most common *spa* types. Only one isolate was assigned to CC8, a clonal lineage linked to high within-herd prevalence of mastitis. A total of 14% (n = 8) of strains were resistant to penicillin and one strain each was oxacillin and pirlimycin resistant. While no clinical breakpoints are available for the combination of kanamycin/ cefalexin, growth of all strains was inhibited by the lowest combination of kanamycin/ cefalexin concentrations tested (4 µg/ml kanamycin and 0.4 µg/ml cefalexin). One strain assigned to CC20, ST389 and t2094 exhibited resistance to penicillin, oxacillin, and pirlimycin as well as intermediate susceptibility to erythromycin and high MICs for several antimicrobial agents, for which no breakpoints were available.

PW375 Characterization of antimicrobial resistance in probiotic and starter cultures

Vita Rozman[1], Petra Mohar Lorbeg[1], Bojana Bogovič Matijašić [1]

[1]*Institute of Dairy Sience and Probiotics, Department of Animal Science, Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia*

Background: Lactic acid bacteria (LAB) and bifidobacteria deliberately introduced into the food chain may act as a reservoir of antimicrobial resistance (AR), which is considered a safety concern.

Objectives: Our aim was to characterize AR of probiotic and starter cultures (n = 20). In addition, a comprehensive *in silico* assessment of the prevalence and flanking sequences of the *tetW* gene (FS*tetW*) across lactobacilli and bifidobacteria (n = 1423) was conducted.

Methods: Minimum inhibitory concentrations (MICs) of 16 antimicrobials were determined using broth microdilution method and/or E-test. Antimicrobial resistance genes (ARG), mutations, FS*tetW,* genomic islands (GIs), and mobile genetic elements (MGE) were predicted *in silico* using different databases and platforms (CARD, Resfams, ResFinder, ARG-ANNOT, FARME DB, IslandViewer4, PlasmidFinder, PointFinder).

Results: Several strains exhibited phenotypic resistance to kanamycin, tetracycline, chloramphenicol, quinupristin-dalfopristin, ciprofloxacin, and neomycin, but these resistances did not always correspond to the presence of ARG and vice versa. Acquired *tetW* gene was detected in four probiotic strains of *Bifidobacterium animalis* subsp. *lactis,* whereas no acquired ARG were identified in genomes of lactobacilli. Nevertheless, homologs of AR proteins were predicted in all proteomes. We found higher prevalence of *tetW*, which is often flanked by MGE, in analysed bifidobacteria (31.9 %) than in lactobacilli (6.3 %). Result suggest that FS*tetW* may be associated with GIs and were conserved in several strains, including potential pathogens. Our findings provide an insight into AR of probiotics and starter cultures with an emphasis on tetracycline and into the safety of these strains in context of AR.

PW376 Characterization of Bacillus cereus group isolates from powdered food products

Heini Nicole[1], Roger Stephan[1], Ehling-Schulz Monika[2], Sophia Johler[1]

[1]*University of Zurich, Institute for Food Safety and Hygiene, Zurich, Switzerland*
[2]*University of Veterinary Medicine Vienna, Functional Microbiology, Institute of Microbiology*

Background: Mashed potato powder as well as powdered infant formula (PIF) are frequently contaminated with *Bacillus cereus sensu lato (B. cereus s.l.*), mainly with its spores. These products have also been implicated in foodborne illnesses.

Objectives: We aimed to chartacterize *B. cereus s.l.* isolates originating from powdered products.

Methods: We performed sporulation assays, toxin gene profiling, and *panC* typing combined with a SplitsTree analysis. Furthermore, cytotoxicity assays with *B. cytotoxicus* isolates were performed.

Results: 78% of PIF tested positive for *B. cereus s.l.*, whereas 92% of all mashed potato powders were positive. In total, 43 isolates were further characterized. The *nhe* and *cytK2* genes were most frequently detected. Moreover, a cereulide-producer was detected from PIF. Most isolates were assigned to *panC* group III, but members of group II, IV, V, and VII could also be found. Nine *B. cytotoxicus* were isolated out of nine mashed potato powders. All *panC* group VII isolates were positive for *cytK1*. Cytotoxicity assays of these nine isolates revealed one highly cytotoxic strain, while all other isolates exhibited no detectable cytotoxicity, underpinning that cytotoxicity of a certain *B. cereus* group strain cannot be deduced from the sole presence or absence of toxin genes.

PW377 Culture-independent detection of Listeria monocytogenes in cheeses

Jana Minarovicova[1], Adriana Veghova[1], Tomas Kuchta[1], Eva Kaclikova[1]

[1]*Food Research Institute, National Agricultural and Food Centre, Department of Microbiology, Molecular Biology and Biotechnology, Bratislava, Slovakia*

Background: Methods based on direct DNA extraction coupled to real-time PCR are candidates for rapid detection and quantification of pathogens in various food products.

Objectives: This study aimed at selection of an efficient method of DNA isolation from cheese and determination of analytical parameters of real-time PCR coupled to it, namely, detection sensitivity for *Listeria monocytogenes* as well as the quantification range.

Methods: Model cheese samples (mozzarella, quargel, parenica, camembert) artificially contaminated with defined concentrations of *L. monocytogenes* were comparatively analysed. Two open-formula liquid-liquid extraction-based procedures and five commercial kits based on solid phase or liquid-liquid extraction were used to extract DNA from model samples. The extracted DNA samples were subjected to real-time PCR with a 5'-nuclease probe targeting *actA* gene.

Results: All seven extraction procedures produced amplifiable bacterial DNA, however, with different detection and quantification limits of the downstream real-time PCR. Among the commercial kits, the best results were obtained with PowerFood Microbial DNA Isolation Kit and DNeasy Mericon Food Kit, which demonstrated detection limits of 2.5×10^2 - 5.0×10^2 CFU/g. As a rule, quantification limits were 2x higher than the detection limits and the response was linear. From the two open-formula procedures, a lower detection limit was determined for phenol-chloroform extraction. Both latter procedures were considerably time-consuming as well as labour-intensive, and produced higher detection limits than the best commercial DNA extraction kits.

PW378 assessment of aflatoxin levels in refined and unrefined vegetable oils in nigeria

Oluwafemi Flora[1;1], Oni Eniola[2;2]

[1]*federal university of agriculture, microbiology, abeokuta, Nigeria*
[2]*federal Unuversity of agriculture nigeria, microbiology, abeokuta, Nigeria*

Background: The presence of Aflatoxins in vegetable oils pose serious challenges to sustainable development in Sub-sahara Africa. Infestation of edible oils by mycotoxigenic moulds and its carcinogenic nature when consumed necessitates the need to screen the oils.

Objectives: This study was carried out to determine the presence of aflatoxin in refined and unrefined vegetable oils, extract and quantify the aflatoxin.

Methods: The quantification was done using High Performance Liquid Chromatography (HPLC).Ten refined and five unrefined vegetable oils of the same batch were purchased from open markets and vegetable oil factories respectively.

Results: Corn oil, Coconut oil, Soya oil, Palm kernel oil, Palm oil and Groundnut oil had the following aflatoxin concentrations respectively; 157ng/kg, 49 ng/kg, 28 ng/kg, 9 ng/kg, 5ng/kg and 4ng/kg while the other four refined oils had no detectable aflatoxin. Comparing the rate at which the toxin (aflatoxin) travel from unrefined oil to refined oil. Unrefined Corn oil, Coconut oil, Soya oil, Palm kernel oil, palm oil and Groundnut oil of the same batch had the following aflatoxin concentrations respectively; 242ng/kg, 102ng/kg, 67ng/kg, 21ng/kg, 9ng/kg, and 10ng/kg. The result indicated unrefined vegetable oil had more aflatoxin load than refined oil. Low level of aflatoxin in the refined oil which is at permissible level according to European Commission is as a result of bleaching during refining. It therefore implies refinery process reduces aflatoxin and this adds advantages to the vegetable oil refineries and to the consumers.

PW379 SDS-PAGE protein fingerprinting as screening method for optimazing detection of diversity in acidification kinetics of starter cultures

Rafael Tabla[1], Isidro Roa[1], José Emilio Rebollo[2], Felipe Molina[2]

[1]*Centro de Investigaciones Científicas y Tecnológicas de Extremadura*
[2]*Universidad de Extremadura*

Background: The traditional use of raw milk in ripened cheese makes the fermentation process dependent on the natural milk contamination with lactic acid bacteria. The microbiological content of the raw milk is usually highly variable, which may lead to cheese alterations such as early blowing. The selection and use of starter bacteria are principal aspects of cheese making, as the flavour of finished cheese is heavily influenced by the starter cultures. To design starter cultures and select candidate strains, it is of value to determine some physiological characteristics, especially their acidifying activity. However, the time and effort required to study the acidification kinetics of a significant part of the lactococci population isolated from a cheese variety would be burdensome.

Objectives: Analyse the relationship between the protein profiles obtained from *Lactococcus lactis* subsp. *lactis* isolated from Ibores cheese and their lactic acid production, an essential technological property in starter cultures.

Methods:

- Lactococcus lactis strains were isolated from a farmhouse starter-free Ibores cheese.
- Acid production was determined in heat-treated reconstituted skimmed milk.
- Identification of strains was performed by SDS-PAGE of cell free extracts.
- Biolog phenotyping microarray was carried out using GP and AN microplates.
- The acidifying activity was characterized by seven parameters.

Results: The analysis of cell protein extracts of 157 *L. lactis* strains by SDS-PAGE yielded around 80 bands per electrophoretic pattern. Five SDS-PAGE protein profiles and six acidification kinectis curves were distinguished and correlated. Thus, selection of native strains could be optimized by protein fingerprinting.

PW380 Enterotoxin production of Bacillus thuringiensis isolates from biopesticides, foods, and outbreaks

Sophia Johler[1], Eva Maria Kalbhenn[2], Heini Nicole[1], Peter Brodmann[3], Sylvia Gautsch[3], Murat Bagcioglu[1;2], Matthias Contzen[4], Roger Stephan[1], Ehling-Schulz Monika[2]

[1]*University of Zurich, Institute for Food Safety and Hygiene, Zurich, Switzerland*
[2]*University of Veterinary Medicine Vienna, Functional Microbiology, Institute of Microbiology*
[3]*Kantonales Labor Basel-Stadt*
[4]*Chemisches und Veterinäruntersuchungsamt Stuttgart*

Background: While the relevance of *Bacillus cereus* as a major cause of gastroenteritis is undisputed, the role of the closely related *Bacillus thuringiensis* in foodborne disease is unclear. A recent EFSA scientific opinion stresses the urgent need for further data allowing for risk assessment, in particular as *B. thuringiensis* is a commonly used biopesticide.

Objectives: We aimed to gain further insights into the hazardous potential of *B. thuringiensis*.

Methods: To this end, 39 *B. thuringiensis* isolates obtained from commercially used biopesticides, food sources, and foodborne outbreaks were characterized by *panC* typing and SplitsTree analysis, toxin gene profiling, FTIR spectroscopic analysis, a cytotoxicity assay screening for enterotoxic activity, and a sphingomyelinase assay.

Results: The majority of the tested *B. thuringiensis* isolates exhibited low or mid level enterotoxicity, and produced either no or low levels of sphingomyelinase, which is reported to act synergistically with enterotoxins Nhe and Hbl. One strain isolated from rosemary was however classified as highly enterotoxic, surpassing the cytotoxic activity of the high-level reference strain by a factor of 1.5. This strain also produced vast amounts of sphingomyelinase. Our study shows that many *B. thuringiensis* biopesticide strains exhibit mid-level cytotoxicity in a Vero cell assay and that some of these strains cannot be differentiated from isolates collected from foods or in association with outbreaks. We demonstrate that the use of *B. thuringiensis* strains as biopesticides can represent a food safety risk, underpinning the importance of assessing the hazardous potential of each strain used.

PW381 Microbiological characterization of Salame Piemonte IGP: starter cultures selection

Irene Franciosa[1], Ilario Ferrocino[1], Valentina Alessandria[1], Kalliopi Rantsiou[1], Luca Cocolin[1]

[1]*University of Turin, DISAFA, Torino, Italy*

Background: Salame Piemonte is a typical fermented sausage of Nord-West of Italy that is preserved by Protected Geographical Indication. Their microbiota is specific of the region or area where they are produced, a management of microbial resources is essential to protect the sensory characteristics of the product.

Objectives: With the aim of the selection of autochthonous starter cultures for this local fermented sausage, the ecology and microbial dynamics during the fermentation process of three different productions from the same factory were evaluated. The study of fermented sausages was carried out by culture-dependent and independent methods.

Methods: Molecular techniques were used for the characterization of the samples and the application of metagenomics approaches helped in the understanding of the correlation between strains and their respective metabolic activities. Physiological characterizations of selected isolates were carried out: about 540 isolates of LAB and CNC were tested for growth rate at different temperatures and under different salt concentrations, acidification capability and for their nitrate reductase activities. Each test was performed in microplates. Lipolytic and proteolytic activity were evaluated using specific agar media by the identification of clear zones. Concerning safety evaluation of selected strains, the antibiotic susceptibility and potential production of biogenic amines by PCR-targeted methods were verified.

Results: After selection of individual strains or strain consortia for inclusion in hand-made product, reduction at a pilot scale at the local producer's facility with one or more strain combinations will be performed.

PW382 Domain analysis of the cell wall proteinase, PrtP, of Lactococcus lactis

Athanasios Tsagkadouras[1], Ida Nynne Laforce[1], Thais Bezerra[1], Egon Bech Hansen[1]

[1]*Technical University of Denmark, National Food Institute, Kgs. Lyngby, Denmark*

Background: Multi domain extracellular cell wall proteinases are widely distributed among prokaryotes. These enzymes provide a multitude of phenotypes ranging from milk fermentation to pathogenicity.

Objectives: To characterize the function of individual proteinase domains, with focus on:

- PrtP interaction with the casein micelle
- specificity of the enzyme
- coupling proteolysis to cellular peptide uptake

Methods: *Lactococcus lactis* strains isolated from camel milk show novel patterns of proteolysis compared to common dairy strains. Shuffling domains among PrtP enzymes allow the mode of action to be determined.

Fermentations in cow and camel milk was used to characterize functionality under natural and relevant conditions. The cellular growth rate and the speed of acidification reveal the overall efficiency of the proteolytic system and proteomic analysis of peptides in the fermented milk reveals enzyme specificity.

Results: The advantage of covalent attachment of the PrtP enzyme was determined by comparison of pairs with and without the cell wall anchor domain. The benefit differs in the two types of milk used in this analysis.

The pattern of casein cleavage and the length of the peptides released could be modified by domain swapping of PrtP.

It has not escaped our attention that the enzyme mechanism of the *Lactococcus* PrtP enzyme has implications for the entire group of subtilisin like proteases containing a PA domain and fibronectin like domains, of which some are virulence factors of pathogenic bacteria. Our results might therefore lead to new ways of fighting disease in addition to innovation in fermented dairy products.

PW383 Assessment of antibiotic resistance and enterotoxygenicity of coagulase-negative staphylococci isolated from milk and goat cheeses in Poland

Martyna Arciszewska[1], Anna Czubkowska[1], Jolanta Grażyna Rola[1]

[1]*National Veterinary Research Institute, Department of Hygiene of Food of Animal Origin, Puławy, Poland*

Background: Coagulase-negative staphylococci comprise many species and subspecies of different pathogenicity and biochemical properties. Recent findings indicate that the pathogenic potential of certain coagulase-negative staphylococci is comparable to that represented by coagulase-positive *Staphylococcus aureus*.

Objectives: The aim of the study was to determine the antibiotic resistance and enterotoxigenicity of coagulase-negative staphylococci (CNS) to estimate the health risk of people consuming raw milk and milk products from untreated goat milk.

Methods: A total of 100 strains, including 91 CNS isolated from goat's milk and 9 CNS from goat cheese, were tested. The identification of staphylococci isolates was performed by morphology of colonies using Baird-Parker agar, biochemical method (Vitek 2) and mass spectrometry (MALDI-TOF MS). Antimicrobial and methicillin resistance of the isolates were determined using Minimal Inhibitory Concentration (MIC) test and PCR methods, respectively. The detection of enterotoxic genes was performed using two multiplex PCR.

Results: Antimicrobial resistance analysis showed that only 16% of CNS were susceptible to all antibiotics tested and 84% were resistant to at least one of them. The highest resistance was observed for tiamulin (38.0%), fusidate (37.0%) penicillin (33.0%) and trimetophrim (27.0%). 22% of CNS showed resistance to only one antibiotic, resistance for two drugs was found in 13% of isolates and 65% of them were multiresistant (3 to 8 antibiotics). No methicillin resistant strains were detected. Only one strain identified biochemically as *Staphylococcus haemolyticus* showed the presence of enterotoxic genes: *seg, seh, sei.* The *sea*, *seb*, *sec, sed, see, ser, sej, sep* genes were not detected.

PW384 Microbial biogeography of leafy vegetables in a farm to fork perspective

Anna Rosberg[1], Julia Darlison[1], Lars Mogren[1], Beatrix Alsanius[1]

[1]*Swedish University of Agricultural Sciences, Biosystems and technology, Alnarp, Sweden*

Background: Outbreaks of food borne illnesses related to consumption of leafy vegetables have increased significantly during the last decades. Shiga toxin-producing *E.coli* (EHEC) which causes hemorrhagic colitis has been identified as the causal agent for some of the major outbreaks across Europe and USA. The severity of the pathogen is related to the low infectious dose needed and its course of disease with a possibly deadly outcome. Contamination of infectious pathogens on leafy vegetables can occur in the entire production chain – from farm to fork.

Objectives: Within the project "Safe Salad" the phyllosphere microbiome of spinach (*Spinacia oleracea*) and rocket (*Diplotaxis tenuifolia*) was mapped through the different stages of production.

Methods: Samples were taken at a commercial farm in the south of Sweden one week before harvest, at harvest, immediately after washing and drying, and at the end of shelf-life of the packed and stored product (both washed and unwashed leaves were stored and sampled). The microbiome was analysed through viable counts using *Enterococci-*, *Enterobacter-*, and *E.coli*-selective agar to screen for possible contamination of infectious pathogens, as wells as general bacteria. Next generation sequencing using the Illumina platform was performed on all samples displaying the biogeography of the phyllopshere microbiome.

Results:

Results of the study show distinct changes in the phyllopshere microbiota related to plant species, time of year for sampling, and at different points in the production chain. Large differences were seen after harvest. Commercial washing of the produce often led to higher bacterial loads.

PW385 Enzymatic properties of aspartate ammonia-lyase isoenzymes from Propionibacterium freudenreichii

Stefan Irmler[1], Marie-Therese Fröhlich-Wyder[1], Daniel Wechsler[1], Helene Berthoud[1], Meral Turgay[1]

[1]*Agroscope, Berne, Switzerland*

Background: The bacterium *Propionibacterium freudenreichii*is is used for the manufacture of Swiss-type cheeses and is responsible for the typical nutty flavor and eye formation. Aspartate ammonia-lyase catalyzes the degradation of aspartate to fumarate and ammonia. Previous reports have demonstrated that specific aspartate-ammonia lyase activity varies between *P. freudenreichii* strains. Strains exhibiting high aspartate ammonia-lyase activity are regarded as ambivalent for cheese production since on the one hand they can enhance flavor formation but on the other hand they can provoke gas production that leads to splits and cracks in the cheese loaf. Interestingly, genome data obtained from the GenBank database showed that the *aspA* gene, which encodes aspartate ammonia-lyase, is ubiquitous present in *P. freudenreichii*. The gene itself shows considerable between-strain heterogeneity.

Objectives: To better understand the link between genotype and phenotype, the specific aspartate-ammonia lyase activity of three isoenzymes originating from *P. freudenreichii* were studied.

Methods: Three *aspA* genes, which encoded isoenzymes of aspartate ammonia-lyases, were cloned into a vector encoding a his-tag and expressed in *Escherichia coli*. The recombinant proteins were purified and their enzymatic properties were studied using photometric assays and HPLC analysis.

Results: The amino acid sequences of the three his-tagged isoenzymes shared between 85 and 95 % identity. All three isoenzymes, which were purified to apparent homogeneity, catalyzed the degradation of aspartate to fumarate and ammonia. Considerable diffenerences were observed regarding the Michaelis-Menten kinetics.

PW386 Sensory Evaluation of Pretreated and Fermented African Yam Bean Seeds As Soup Condiment

Abimbola Adefiranye[1;2;3], Ekaette N. Dike[4], Joy Ehiwuogu-Onyibe[4], Jane N.C. Okafor[5], Frank A. Orji[4], Samuel O.A. Olatope[4], Mojisola T. Kehinde[4], Victoria T. Olonade[5], Olakunle D. Teniola[6], Gloria N. Elemo[7]

[1]Federal Institute of Industrial Research Oshodi (FIIRO) Lagos, Nigeria., Department of Biotechnology, , Lagos, Nigeria
[2]University of Lagos Nigeria, Cell Biology and Genetics, Lagos, Nigeria
[3]International Institute of Tropical Agriculture (IITA), Genetic Resources Centre, Ibadan, Nigeria
[4]Federal Institute of Industrial Research Oshodi (FIIRO) Lagos, Nigeria., Department of Biotechnology, Lagos, Nigeria
[5]Federal Institute of Industrial Research Oshodi (FIIRO) Lagos, Nigeria., Food Technology, Lagos, Nigeria
[6]Ondo State University of Science and Technology, Okitipupa, Ondo State, Nigeria., Department of Biological Sciences, Ondo, Nigeria
[7]Federal Institute of Industrial Research Oshodi (FIIRO) Lagos, Nigeria., Directorate, Lagos, Nigeria

Background: An experiment was carried out on sensory evaluation of pre-treated and fermented African yam bean (*Sphenostylis stenocarpa*) seeds as soup condiment at the Federal Institute of Industrial Research, Oshodi Lagos, Nigeria. Sensory evaluation and consumer acceptability of the sample of African locust bean (*Parkia biglobosa*) i.e. a positive control was compared to three pre-treated and fermented (soaked, boiled and roasted) samples of African yam bean, and Soup only (a negative control).

Objectives: This is to determine the consumer acceptability of the best pre-treatment method to adopt for fermented African yam bean condiment and comparing these with a known condiment by known native users

Methods: The Assessments were under aseptic conditions. The responses evaluated by questionnaires. Spider web graphic analysis was plotted using 9-Point Hedonic scale to determine consumers' preferences.

Results: From the analysis, soup from the positive control was mostly accepted; with a mean rank of 7.54, followed by the soup only with mean rank of 7.50. Soup from the roasted was preferred to the soup from the soaked and boiled African yam bean with mean rank of 7.41. There was no significant ($P>0.05$) difference in the ranking of the roasted African yam bean with the positive and negative control. Although, soup from the fermented African locust bean was preferred to African yam bean, however, soup from the roasted African yam bean seeds was a comparable alternative.

PW387 Comparative genomics and growth characterisation of beer and pickle spoilage Pectinatus strains shows no clear difference in tolerance to brewing-related stresses

Timo Kramer[1], Philip Kelleher[2], Julia van der Meer[3], Tadhg O' Sullivan[4], Jan-Maarten A. Geertman[4], Sylvia H. Duncan[1], Harry J. Flint[1], Petra Louis[1]

[1]*The Rowett Institute, Abereen, United Kingdom*
[2]*University College Cork, APC Microbiome, Cork, Ireland*
[3]*Genedata AG, Basel, Switzerland*
[4]*Heineken Supply Chain B.V., Zoeterwoude, Netherlands*

Background: The genus *Pectinatus* currently consists of five recognized species, three of which are associated with beer spoilage and two with pickle spoilage. These organisms have only been isolated from industrial settings and have no known natural reservoir.

Objectives: To characterize the genetic and physiological diversity within the genus *Pectinatus*, specifically focussing on brewery-relevant stress reponse mechanisms.

Methods: Whole genome sequencing of 13 isolates of five species of *Pectinatus* and comparative genomic analysis coupled with anaerobic culturing of a selection of these organisms under brewery and pickling specific stress conditions.

Results: Genomic analysis revealed high degrees of conservation within all strains of *Pectinatus frisingensis*. Main differences were observed in carbon source utilization. Small yet distinct genetic differences were found between beer spoilage associated and pickle spoilage associated species. These differences could be attributed to the presence of genes coding for halotolerance, carbon source utilization, associated transporters and regulatory genes. Few physiological stress response differences between beer spoilage and pickle spoilage-associated species were detected. However, in accordance with the genomic findings, pickle spoilage-associated species exhibited higher salt tolerance. No clear difference in maximum growth rate and culture density was observed in response to brewery-specific stress when comparing beer and pickle spoilage-associated species. This indicates that the pickle spoilage-associated organisms may pose novel potential hazards for brewing environments.

PW388 In situ production and characterization of cloud forming dextrans in fruit juice-base media

Viktor Eckel[1], Rudi F. Vogel[1], Frank Jakob[1]

[1]*Lehrstuhl für Technische Mikrobiologie, Technische Universität München, Freising, Germany*

Background: Turbidity in beverages is typically achieved by addition of emulsion based cloud systems. Their intrinsic instability necessitates the widespread use of technological measures and use of food additives to prevent emulsion decay.

Objectives: In this work we explored the possibility to establish a new generation of natural, stable clouding systems based on bacterial dextrans.

Methods: *Lactobacillus hordei* TMW 1.1907 was used to produce dextrans by fermentation of fruit juice based media. Asymmetric flow field-flow fractionation coupled to multi-angle laser light scattering was used for determination of molecular weight and rms radii.

Results: By varying the fermentation conditions, two types of dextran molecules could be produced at yields of 2.5-8.5 g/L. These molecules were stable at pH 3, against heat treatment (85°C, 3 min) and upon long-term storage, showing strong, unchanged turbidity after 3 months storage. Fermentations at native fruit juice pH of 3.3 and 3.4 showed low dextran yield, while adjustment to pH 7 increased yields. Molecular weight and rms radius of produced dextran molecules was higher in fermentations carried out at 20 °C than at 30 °C. RMS radii of dextrans was 66.0 to 87.4 nm, while the molecular weight was determined to be in the range of 103.1 to 141.6 mDa. Our data shows the suitability of fruit juice based media for *in situ* production of dextrans and the applicability of these dextrans and fermentation products as cloud forming food additives. This opens up new applications for dextrans as stable cloud forming food additives produced *in situ*.

PW389 A diverse microbial community in Teff fermentation contributes to the sensory and antimicrobial properties

Yared Bezabhe[1;2], Jana Jass[2], Solomon Gebre selassie[1], Abraham Aseffa[3]

[1]Addis Ababa University, Microbiology, Immunology and Parasitology, Addis Ababa, Ethiopia
[2]Örebro University, School of Science and Technology, the Life Science Center-Biology, Örebro, Sweden
[3]Armauer Hansen Research Institute, Addis Ababa, Ethiopia

Background: Ethiopia ranks fifth among countries with the highest annual child death due to diarrhea according to the WHO. This is mainly due to contaminated food and water. A fermented Teff sourdough pancake known as Injera is a staple food for most Ethiopians. Pathogens may be introduced into the sourdough due to poor hygiene and contaminated water. While it is believed that the fermenting microbiota outcompetes and eliminates contaminating pathogens, definitive data are lacking.

Objectives: The study aimed to investigate the microbial diversity and dynamics of traditional Teff fermentation and the possible role in sensory and antimicrobial properties.

Methods: The study aimed to investigate the microbial diversity and dynamics of traditional Teff fermentation and the possible role in sensory and antimicrobial properties.

Results: We have shown that a diverse microbial community was involved in Teff fermentation irrespective of the fermentation cycle. The microbial population changed as the fermentation progressed, however, several *Lactobacillus* species along with *Candida humilis* and *Pichia occidentalis* dominated at each stage of the fermentation. Presumptive *Lactobacillus* species involved in the fermentation also showed antimicrobial activity against enteric pathogens, suggesting possible application in the preparation of standard Injera. This study indicates that standardized Teff fermentation with selected microorganisms could be used to introduce health benefiting functional foods.

PW390 Multilocus sequence types and antimicrobial resistance of Campylobacter jejuni isolates from different sources in Lithuania

Jurgita Aksomaitiene[1], Sigita Ramonaite[1], Aleksandr Novoslavskij[1], Mindaugas Malakauskas[1]

[1]*Lithuanian University of Health Sciences, Department of Food Safety and Quality, Kaunas, Lithuania*

Background: The most frequently reported foodborne *Campylobacter* infections are in most cases self-limiting and antimicrobial treatment is necessary only in severe prolonged cases. However antimicrobial resistance of *Campylobacter* has increased dramatically in addition to emerging of multidrug resistance.

Objectives: The aim of our study was to determine the minimum inhibitory concentration of the antimicrobials for *C.jejuni* and analyse the overlapping by MLST and antimicrobial resistance of *C. jejuni* among isolates collected from different sources.

Methods: In total 341 *C.jejuni* isolates from children clinical cases (n=101), broiler products (n=98), dairy cattle (n=41) and wild birds (n=101) were genotyped by MLST and tested against phenotypic resistance to ciprofloxacin, tetracycline, gentamycin, ceftriaxone and erythromycin by agar dilution method. MIC were compared with MLST types to find possible associations between ST and resistance.

Results: Resistance to ciprofloxacin, tetracycline and ceftriaxone was found in 91.5%, 37.8%, and 60.4%, of the tested *C. jejuni* isolates, respectively. Only three isolates assigned (ST-5, ST-19, ST-5843) were resistant to erythromycin (0.9%). Meanwhile, all *C.jejuni* isolates were sensitive to gentamycin. A total of 93 isolates (27.3%) were resistant to three classes of antibiotics and were considered as multidrug resistant. We identified 10 *C. jejuni* specific antimicrobial resistance profiles and most of the examined *C. jejuni* isolates (80.6%) showed resistance to one of three profiles: CIP+AXO (28.2%), TET+CIP+AXO (26.7%) and CIP (25.8%). The TET+CIP+AXO multidrug resistance profile was confirmed for majority of the examined *C.jejuni* isolates assigned to ST-21 (77.8%), ST-3098 (80%), ST-354 (100%), ST-464 (53.3%), ST-6411 (66.7%) and ST-6391 (100%).

PW391 Factors influencing decarboxylase activity of Enterococcus

Pavel Pleva[1], Leona Buňková[1], Eva Lorencová[2], František Buňka[2]

[1]Tomas Bata University in Zlín Faculty of Technology, Department of Environmental Protection Engineering, Zlín, Czech Republic
[2]Tomas Bata University in Zlín Faculty of Technology, Department of Food Technology, Zlín, Czech Republic

Background: Biogenic amines are produced by decarboxylase activity of microorganisms in foodstuff. Their consumption can adversely affect human health. Therefore, the aim of this study was to determine the influence of factors on decarboxylase activity of enterococci.

Objectives: In this work, the ability to produce 8 biogenic amines (phenylethylamine, histamine, cadaverine, putrescine, tryptamine, tyramine, spermidine, and spermine) by *Enterococcus* strains isolated from rabbit meat (*Oryctolagus cuniculus*) was investigated. Additionally, the effect of some factors such as temperature (6, 12, 30°C), pH (5, 6, 7), and NaCl (1, 2, 3, 6%) on decarboxylase activity of enterococci was tested.

Methods: The kinetics of biogenic amines formation was monitored during cultivation of bacteria prepared by derivatization with dansyl chloride. The production of eight biogenic amines was monitored by HPLC system equipped with a binary pump; an autosampler (LabAlliance, USA); a column thermostat; an UV/VIS DAD detector (λ = 254 nm); and a degasser (1260 Infinity, Agilent Technologies, USA).

Results: The obtained results showed that all studied enterococci produced predominantly tyramine and putrescine. Putrescine (>100 $mg.l^{-1}$) was produced by two strains of Enterococcus faecium. The most significant influence on biogenic amines production had temperature. The highest tyramine production was observed at 30°C after 24 hours (*Enterococcus faecium* 2201 $mg.l^{-1}$, *Enterococcus* sp., 1700 $mg.l^{-1}$). Enterococci produced a substantial amount of tyramine and phenylethylamine at pH 5 - 6, with the highest production of mentioned amines at 30°C and pH 6. In conclusion, enterococci are significant producers of tyramine in food.

PW392 Monitoring the photocatalytic activity of a TiO2 nanoparticles based surfactant against biofilms formed by foodborne bacteria

Agapi Doulgeraki[1], Christina Kamarinou[1], Anthoula Argyri[1], Chrysoula Tassou[1], George-John Nychas[2], Nikos Chorianopoulos[1]

[1]*Institute of Technology of Agricultural Products, Hellenic Agricultural Organization - DEMETER, Lycovrissi, Greece*
[2]*Laboratory of Microbiology and Biotechnology of Foods, Agricultural University of Athens (AUA), Department of Food Science and Human Nutrition, Athens, Greece*

Background: Both pathogenic and spoilage microorganisms adhere to the surfaces in food processing environments, survive, grow and form biofilms. Microbial interactions play an important role in the initial cell adhesion and endurance of biofilm to disinfectant stresses.

Objectives: To evaluate the disinfecting activity of an innovative photocatalytic surfactant based on TiO_2 nanoparticles, against various biofilms.

Methods: *Salmonella* Enteritidis, *Listeria monocytogenes* and *Escherichia coli* were left to form biofilm in mono- or co-cultures with food spoilage bacteria on stainless steel (SS) coupons immerged in TSB at 20°C for 6 days. The spoilage bacteria used for co-cultures were belonged to the genera *Leuconostoc*, *Lactobacillus*, *Serratia*, *Citrobacter*, *Hafnia*, *Proteus*, *Pseudomonas* and *Brochothrix.* After biofilm formation, the SS coupons were immersed in the disinfectant and afterwards exposed to ultraviolet radiation for 2h for each side separately. Biofilm population was enumerated by bead vortexing-plate counting method.

Results: It was evident that the exposure to ultraviolet radiation reduced the population of biofilm cells below the detection limit of the method in most cases, whereas the use of the disinfectant strengthened the antimicrobial activity of UV. Furthermore, it was observed that the presence of more than one species affected both cell biofilm ability and their resistance to ultraviolet radiation and disinfectant. In conclusion, the use of the surfactant with the photocatalytic TiO_2 agent as an alternative way of cleaning contaminated surfaces presents an intriguing case that may provide powerful solutions regarding biofilm disinfection within the food processing environments.

PW393 Novel teachnologies for prevention and treatment of bacterial biofilms in the Food Industry

Apurva Patange[1], Brijesh Tiwari[1]

[1]*Teagasc Food Research Centre, Department of Food Chemistry and Technology, Ireland*

Background: Biofilm are persistent form of bacteria known to attach to food surfaces while some internalise within the matrix which often complicates or negates food disinfection process. Non-thermal plasma and acoustic airborne are emergent technologies which have attracted attention for its enhanced microbial safety, non-thermal nature and fast processing.

Objectives: The objective of this study was to investigate the effect of atmospheric pressure cold plasma and airborne acoustic technology on bacterial biofilms associated commonly associated with food industry.

Methods: Bacterial biofilms in mono- or mixed species biofilms. The biofilm populations were treated with ACP and airborne acoustic for 60-300s and subsequently stored for 0-24h. The inactivation efficacy was evaluated in terms of reduction of culturability and retention of metabolic activity using colony count and XTT assay.

Results: Microorganisms showed varying susceptibility to ACP treatment. A strong effect of process and treatment parameters was observed. ACP treatment reduced bacterial biofilms by 3 Log CFU/ml while Airborne acoustic treatment was able to reduce microbial population by 2 Log cfu/ml. Mixed biofilm cultures showed higher resistance and required longer treatment times. These non-thermal technologies shows potential as an efficient control mechanism against bacterial biofilm contamination which could help develop a new sustainable technology to solve serious economic and health issues.

PW394 Assessing the effects of four different egg disinfection methods on hatching broiler eggs-field trial

Gerzon Motola[1], Wiebke Tebrün[2], Michael Pees[2], Josef Bachmeier[3], Hafez Hafez Mohamed[1], Sarah Brüggemann-Schwarze[1]

[1]*Freie Universität Berlin, Institute of Poultry Diseases, Berlin, Germany*
[2]*University of Leipzig, Clinic for Birds and Reptiles, Leipzig, Germany*
[3]*BWE-Brüterei Weser-Ems GmbH & Co., Brüterei Süd ZN, Regenstauf, Germany*

Background: Bacterial contamination in poultry products is of major concern for the poultry industry and consumers. The present study focusses on the reduction of bacteria on eggshells at the beginning of the broiler production chain. Four disinfection methods were selected for this field study, based on previous *in vitro* and *in vivo* data from laboratory disinfection trials against ESBL (extended-spectrum beta-lactamase) producing *E. coli.*

Objectives: Four different disinfection methods were tested in a field trial for a) their efficacy on the eggshell and effects on b) hatching rate, c) meconium microbiota and d) performance.

Methods: Five groups were tested 1) Formalin fumigation, 2) H_2O_2 + alcohol, 3) peracetic acid and 4) low energy electron beam, 5) non-disinfected control group. Each contained 2 000 - 54 000 eggs, depending on the complexity of the disinfection. Eggs were incubated, chicks hatched and were housed separately under normal field conditions. a) Disinfection efficacy was determined using egg wash samples comparing non-treated and disinfected groups. b) The hatching rate was analyzed for all groups. c) Meconium pool samples of newly hatched chicks were collected separately for all groups. d) The flock performance was compared with respect to i.e. weight gain. All samples were examined for total bacterial count, ESBL producers and MRSA.

Results: Formalin fumigation, peracetic acid and low energy electron beam reached a 2-fold log reduction (and more) in total bacterial counts. The hatching rate was comparable. Detailed results on meconium composition of hatched broiler chicks will be presented along with performance data.

PW395 Characterisation of antifungal molecules produced by starter cultures using a QuEChERS method applied to dairy products

Meg Rouxel[1], Pierre Marchand[2], Camille Juin[1], Philippe Blanc[2], Julien Verdon[1], Jean-Marc Berjeaud[1], Willy Aucher[1]

[1]*Ecologie et biologie des interactions UMR CNRS 7267, Microbiologie de l'eau, Poitiers, France*
[2]*Bioprox, Noyant, France*

Background: Food products are nutrient-rich matrixes susceptible to be spoiled by fungi. These contaminations by molds or yeasts are responsible for considerable food waste (estimated between 5% and 10% of the world's food production) and economical losses. To limit fungal growth in food product, technical processes are applied such as cold storage or addition of chemical preservatives. For the last decades, consumers have been rejecting additives. Therefore, the demand for naturally preserved food products has grown. The utilisation of bioprotective cultures is an alternative to chemical preservatives, especially in dairy products.

Objectives: The aim of this study is to select lactic acid bacteria (LAB) strains with strong antifungal activity and to characterise their antifungal compounds produced in dairy products. The selected strains may be used as bioprotective cultures.

Methods: Antifungal activity of 18 LAB strains were screened in MRS agar media by the overlay method. Four strains have a strong activity against yeasts and among them, two also exhibit an activity against molds. Both ability to grow and antifungal activity were tested in milk media. Characterisation and quantification of antifungal compounds produced in fermented milk were achieved by HPLC and by the QuEChERS method coupled to LC-MS.

Results: Some antifungal compounds produced were identified. They are mainly aromatic organic acids (benzoic acid, phenyllactic acid,) which have a synergistic effect. Currently, different combinations of these strains are tested for their potential use as bioprotective cultures.

PW396 Synthesis of oligosaccharides by Leuconostoc mesenteroides 20193 and Weissella confusa A16 by fermenting Brewers' spent grain and malt syrups

Prabin Koirala[1], Yaqin Wang[1], Ndegwa Henry Maina[1], Kati Katina[1], Rossana Coda[1]

[1]*University of Helsinki, Food and Nutrition, Helsinki, Finland*

Background: Oligosaccharides (OS) are carbohydrates made of 3 to 10 units of simple sugars, they are synthesized by lactic acid bacteria (LAB) and have numerous biological functions. There is a direct influence of the type of substrate, LAB and fermenting conditions on *in vitro* synthesis of OS. Creating new functionality into under-utilized, food-grade raw material using fermentation is a tool to increase their employment in novel food applications. The study will explore how the variation in nutritional compositions of substrates and fermenting conditions could prompt bacteria to synthesize different types of OS.

Objectives: To characterize different types of OS synthesized by *Lecuconostoc mesenteroides* 20193 and *Weissella confusa* A16 by fermenting brewers' spent grain (BSG) and malt syrups (MaS) in varying conditions and to observe their overall influence upon substrate's technological properties.

Methods: LAB were used to ferment BSG and MaS. For quantification and structural characterization of OS, High performance liquid chromatography, Liquid chromatography–mass spectrometry and Nuclear magnetic resonance spectroscopy were used.

Results: The variation in quantity of OS was observed immediately with higher structural diversity. Besides different OS, fermentation improved the technological properties of the substrates for other applications significantly. There is a link between structural variability among OS and their roles in improving the substrate's technological and nutritional properties. Thus, elucidating the regulatory mechanism behind the synthesis of OS by LAB in different fermentation conditions has a very high potential for improving the current methods to obtain desired OS and increase their use in food applications.

PW397 Real-time PCR for detecting the inv A and ttr genes of Salmonella spp. in animal feed

Marko Dmitrić[1], Dejan Vidanovic[2], Kazimir Matovic[2], Zoran Debeljak[3], Nedjeljko Karabasil[4]

[1]*Veterinary Specialized Institute Kraljevo, Department of Food Safety, Kraljevo, Serbia*
[2]*Veterinary Specialized Institute Kraljevo, Department of Laboratory Diagnostic, Kraljevo, Serbia*
[3]*Veterinary Specialized Institute Kraljevo, Department of Epizootiology, Kraljevo, Serbia*
[4]*University of Belgrade, Faculty of Veterinary Medicine, Department of Food Hygiene and Technology, Belgrade, Serbia*

Background: The microbiological standard method for the detection of *Salmonella* spp. in animal feed requires at least four days for final confirmation and consequently, there is a need for an alternative rapid methodology for its detection.

Objectives: The aim of this study was to compare two open-formula, diagnostic real-time PCR methods for detecting the *inv A* gene and *ttr* gene *Salmonella* spp. in animal feeds, after DNA extraction using the Chelex resin.

Methods: At the same time, *Salmonella* spp. have been detected using the standard method (EN ISO 6579-1:2017) and real-time PCR protocols for the detection of *inv A* and *ttr gene Salmonella* spp., with certain modifications described in our previously published study.

Results: The results of this study have been satisfactory while applying both real-time PCR protocols. The relative sensitivity for the alternative methods and the relative trueness were both 100%. No false negative results were detected. Lower Ct values were obtained using real-time PCR protocol for detecting the *ttr* gene. The results were compared in total of 100 naturally and artificially contaminated feed samples to those of the EN ISO 6579-1:2017 standard method. The whole procedure of real-time PCR methods in this study was less than 24 h, in contrast to 4–5 days of analysis time for the standard method (EN ISO 6579-1:2017).

PW398 Butyrate effect on extracellular GABA production in Listeria monocytogenes 10403S WT and Lactic Acid Bacteria isolates

Carolina Bruschi [1], Kimon Andreas Karatzas[1]

[1]*University of Reading, Food and Nutritional Sciences, Reading, United Kingdom*

Background: Butyric acid is extensively used in food, beverage, pharmaceutical, and chemical industries. Butyrate is also produced by bacterial fermentation of carbohydrates in the colon. Gamma-aminobutyric acid (GABA) is an inhibitory neurotransmitter, and several studies have associated GABA with health benefits, including decreased anxiety, hypotension, and autoimmune inflammation suppression. Different bacteria have been reported to produce GABA as a product of glutamate decarboxylase system, mechanism that helps bacteria survive in acidic environments.

Objectives: This work aims to evaluate the effect of sodium butyrate on extracellular GABA (GABAe) production of isolates from cheese, gut, and *Listeria monocytogenes* 10403s WT.

Methods: Overnight cultures of 11 cheese and 16 gut isolates were sub-cultured in 20ml MRS broth with 3g/L glutamate with 40mM sodium butyrate and pH adjusted to 6. Cultures were incubated at 37°C/48h anaerobically and samples taken for GABAe analysis. *L. monocytogenes* 10403s WT overnight culture was inoculated in 20ml BHI broth with 20mM sodium butyrate in 250 ml conical flasks and incubated at 37°C/24h/120RPM aerobically, or in 50ml falcons and incubated 37°C/48h anaerobically. pH was reduced with 1M HCl to 4.3 for GABAe production analysis, and to 3 for acidic survival. Cultures were incubated at 37°C/60min. Viable cell counts were assessed every 20 min and GABAe samples were obtained after 60min.

Results: All cheese and 92.59% of gut isolates presented reduced GABAe production after growth on media with sodium butyrate. *L. monocytogenes* 10403S WT grown with sodium butyrate showed a decrease in GABAe production and was more sensitive during acid stress.

PW399 Microbial community dynamics of different types of mabisi, a traditional fermented milk

Bernard Moonga[1;2], Sijmen Schoustra [1;2], Anita Linnemann[1], John Shindano[2], Eddy Smid[1]

[1]*Wageningen University, Laboratories of Food Microbiology, Food Quality Design & Genetics, Wageningen, Netherlands*
[2]*University of Zambia, Department of Food Science & Nutrition, Lusaka, Zambia*

Background: Spontaneous fermentation is employed in certain artisanal products and has a bearing on microbial communities of the product. One such product is mabisi which is made by allowing raw milk to ferment spontaneously at room temperature in a calabash (gourd), plastic or metal container for about 48 hours and there after it is stirred before consumption.

Objectives: The aim of this study was to investigate the effect of different production methods on the microbial communities of mabisi.

Methods: Four different production methods were used to produce four types of mabisi, namely; tonga, illa, backslopping and barotse. The first three types were produced traditionally in plastic containers by four different producers in their home settings while the barotse type and another tonga type were produced in the laboratory under controlled conditions. And the end samples were analysed for microbial community profiles using 16S RNA as well as physiochemical properties.

Results: We found that tonga mabisi had more diverse microbial communities regardless of producer or site of production compared to the illa, backslopping and barotse types. The dominant lactic acid bacteria (LAB) genus for these three were mainly *Lactobacillus* and *Lactococcus* while only the latter was the dominant LAB in tonga type. The appearance and consistency of these products was distinct and different but the pH was similar and only differed when elevated or low fermentation temperatures were used.

PW400 Detection and analysis of carbapenemase genes on the surface of commercially-available spices

Minako Mochizuki[1], Miyuki Nakatani[2], Mariya Ueda[2], Shiori Nakaoka[2], Sumio Maeda[1;2]

[1]Nara Women's University, Graduate School of Humanities and Sciences, Nara, Japan
[2]Nara Women's University, Faculty of Human Life and Environment, Nara, Japan

Background: The spread of multi-drug-resistant bacteria, particularly those producing carbapenemases, has become a major public health concern. The presence of carbapenemase genes has been mainly reported in clinical samples, whereas the presence of carbapenemase genes in commercially-available foods has been insufficiently studied.

Objectives: This study aimed to detect carbapenemase genes (bla_{NDM}, bla_{IMP}, bla_{KPC}, and $bla_{OXA\text{-}48\ like}$) in various commercially-available spices, and to characterize any that were found to be present.

Methods: Imported spice samples (13 species) were purchased during 2017–2018 from grocery stores in Japan. DNA was collected and purified from the surface of these samples. PCR of the purified DNA was conducted using the primers for each carbapenemase gene. PCR products obtained were sequenced and analyzed.

Results: At least 9 PCR products were obtained from 29 spice samples. They were identified as parts of independent carbapenemase genes. Some of them appeared to be candidates for novel carbapenemase variants. Our results indicate that carbapenemase gene DNA is commonly present on the surface of commercially-available spices. These spices originate from various countries, suggesting the possibility that carbapenemase genes may be spreading worldwide in association with the trade and distribution of food.

PW401 Low pressure plasma to decontaminate Listeria innocua in Taleggio PDO cheese: Feasibility and preliminary outcomes

Tiziana Silvetti[1], Matteo Pedroni[2], Stefano Morandi[1], Espedito Vassallo[2], Ivano De Noni[3], Milena Brasca[1]

[1]*Institute of Sciences of Food Production, Italian National Research Council, Milan, Italy*
[2]*Institute of Plasma Physics, Italian National Research Council, Milan, Italy*
[3]*University of Milan, Department of Food, Environmental and Nutritional Sciences, Milan, Italy*

Background: Foodborne Listeriosis is a major public health concern worldwide, leading to higher hospitalization and fatality rates in comparison to other microbial pathogens.

Soft mould- and smear-ripened cheeses are very sensitive to infection with *Listeria monocytogenes*, and their rinds are particularly favourable sites for listerial multiplication.

Unlike other technologies effective in controlling food pathogens, non-thermal low pressure plasma could impair listerial growth without or with limited effects on the sensory properties of cheese.

Objectives: This study aimed at assessing the application of low pressure plasma processing for decontaminating *Listeria innocua* in rinds of Italian Taleggio PDO cheese. The effect of the plasma treatment on rind color was investigated as well, since this parameter represents a typical quality attribute of this soft smear-ripened cheese.

Methods: The closely related non-pathogenic species *L. innocua* was employed instead of *L. monocytogenes* for artificial contamination of Taleggio rinds. Two *L. innocua* strains were mixed in equal ratios and spread (5.56 $\log_{10}$ CFU) on rinds. Samples were exposed to Ar-O_2 low pressure plasma (85-145 W, 7 mbar) at different time intervals. *Listeria* was enumerated (ISO 11290-2:1988). Color measurements were performed with a tristimulus colorimeter and expressed in terms of CIELAB color space values.

Results: Low pressure plasma treatment was effective for inhibiting *L. innocua*. The logarithmic reductions depended on the exposure time and the highest inactivation (1.33 Log_{10} CFU/g) was observed after 20 min treatment. The treated rinds complied with the product specification concerning rind color and showed a less yellow, higher color intensity.

PW402 Himanthalia elongata applications in foods: a microbiological perspective

Francesco Martelli[1], Claudia Favari[1], Pedro Miguel Mena Parreno[2], Daniele Del Rio[2], Erasmo Neviani[1], Valentina Bernini[1]

[1]*University of Parma, Department of Food and Drug, Parma, Italy*
[2]*University of Parma, Department of Veterinary science, Parma, Italy*

Background: *Himanthalia elongata* is a brown oceanic seaweed rich in bioactive compounds and could have an important role in food production because of the antimicrobial and antioxidant properties of its extracts. In addition seaweeds fermentation by lactic acid bacteria could be a strategy to obtain new fermented functional foods. During fermentation high-molecular-weight organic compounds are metabolized into relatively simple substances. This can improve their bioavailability and also change the phenolic profile and the antimicrobial properties of the substrate.

Objectives: The study aimed to evaluate different application of seaweeds in food production. For this reasons were evaluated: i) the ability of lactic acid bacteria to ferment the substrate; ii) the antimicrobial and antioxidant activities of *H. elongata* extracts before and after fermentation and following an high pressures treatment.

Methods: Three strains belonging to *Lactobacillus casei* group (*Lactobacillus casei, Lactobacillus paracasei* and *Lactobacillus rhamnosus)* and a *Bacillus subtilis* strain were used for the solid state fermentation. An HPP treatment was also performed (6000 bar, 5 minutes, 5°C). Seaweed extraction was carried out with ethanol, water (70,30%) and 0,1% formic acid. Bacterial growth was monitored by plate counts and the antimicrobial activity of the extracts was tested against the main food pathogenic bacteria.

Results: *H. elongata* allowed for the growth of *Lactobacillus casei* group bacteria and *Bacillus subtilis*, showing the fermentability of the substrate. An interesting antimicrobial activity, in particular against *Listeria monocytogenes*, was revealed by the not fermented extracts. During fermentation and following the HPP treatment the antimicrobial compounds were reduced.

PW403 Assessment of bacterial level and diversity on different broiler carcass areas processed in different slaughterhouses

Zhongjia Yu[1], Kurt Houf[1]

[1]*Ghent University, Veterinary Public Health and Food Safety, Merelbeke, Belgium*

Background: bacterial contamination on food products is associated with food spoilage and foodborne infections. For broiler carcasses, different initial levels of contamination on the living birds, and different slaughter procedures may result in unequally dispersed bacterial contamination levels and diversity of poultry products.

Objectives: determination of the bacterial contamination level and population on three areas of broiler carcasses and assess the potential association of the farm and slaughterhouse of processing.

Methods: classical ISO-based isolation methods combined with MALDI-TOF MS identification as well as 16S amplicon sequencing were applied to examine the bacterial contamination level and diversity of neck, breast and back skin of 20 broiler carcasses from four slaughterhouses and two types of poultry farms.

Results: between the three areas, total aerobic bacterial levels were not significantly different, though the number of anaerobic bacteria was higher on the neck skin ($p<0.05$). The levels of lactic acid bacteria and *E. coli* were significantly slaughterhouse dependent. *Pseudomonas* was most abundant. The bacterial diversity turned out not to be area-dependent, though slaughterhouse and farm-type dependent. Different slaughterhouses yielded their own microbiome on the skin of chicken carcasses.

Conclusions: during slaughter, unequal exposure of the different areas of a broiler carcass has limited impact on the total aerobic bacterial level, though both poultry farm and slaughterhouse are important factors to shape initial bacterial levels and diversity on poultry products.

PW404 Unravelling the microbiome of Sesotho (traditional Lesotho sorghum beer) through targeted metagenomics

Errol D. Cason[1], Angel Valverde[1], Bennie Viljoen[1], Jan-G Vermeulen[1], Marcele Vermeulen[1], Laurinda Steyn[1], Olga De Smidt[2], Mpho Malefane[2]

[1]University of the Free State, Department of Microbial, Biochemical and Food Biotechnology, Bloemfontein, South Africa
[2]Central University of Technology, Centre for Applied Food Security and -Biotechnology, Bloemfontein, South Africa

Background: Indigenous cereal-based fermented foods and beverages constitute one of the main dietary components in Lesotho. This indigenous technology is part of the cultural norm and is practised at house-holds for family consumption, cultural ceremonies and village-level commercial purposes. Due to lack of food preservation facilities such as refrigeration, fermented food products in Lesotho are likely to remain an integral part of the food supply. However, according to available information, very little research has been done on the microbial diversity of traditional fermented foods of Lesotho.

Objectives: To better understand the microbial diversity (both yeast and bacteria) of the fermentation process of Sesotho, a popular fermented beer in Lesotho.

Methods: Samples were collected from five districts in Lesotho. The microbial shifts during the fermentation of Sesotho was elucidated by obtaining samples at 5 key points during the fermentation process. ITS (fungi) and 16S rRNA (bacteria) metagenomics amplicon sequencing was performed using Illumina MiSeq.

Results: Various wild yeasts and lactic acid bacteria from the ingredients and the beer pots was present and contributed to the flavour characteristics of the beer as well as inhibit pathogenic microorganisms. Definitive shifts in the yeast and bacterial diversities throughout the brew process could be observed. There is therefore a need to identify the diversity of microorganisms in Sesotho beer. This research will thus generate vital indigenous knowledge that will form part of a comprehensive database within the ethnic food fermentation fraternity of Lesotho, contributing to socio-economic development and sustainability of the traditional fermented beverage industry.

PW405 Fungal community in Austrian Vorarlberger Bergkäse during ripening

Monika Dzieciol[1], Stephan Schmitz-Esser[2], Lukas Glanz[1], Benjamin Zwirzitz[3], Martin Wagner[1;3], Stefanie Urimare Wetzels[1;3], Evelyne Mann[1]

[1]*Institute for Milk Hygiene, University of Veterinary Medicine, Department for Farm Animals and Veterinary Public Health, Vienna, Austria*
[2]*Iowa State University, Department of Animal Science, Ames, IA, United States*
[3]*FFoQSI, Austrian Competence Centre for Feed and Food Quality, Safety and Innovation, Tulln, Austria*

Background: Vorarlberger Bergkäse (VB) is an artisanal raw milk washed-rind hard cheese (manufactured in Western Austria) without adding external ripening cultures and has a protected designation of origin (PDO). In previous studies, the bacterial and fungal composition of the rind microbiota of VB was assessed by using 16S and 18S rRNA cloning and Sanger sequencing, revealing a high diversity on the VB cheese rind (Schornsteiner et al., 2014).

Objectives: The aim of this study was to identify cheese-associated eukaryotes (yeast and filamentous fungi) present in the VB cheese rinds during ripening process.

Methods: Cheese rind samples (n=200) were taken from ripening cellars of two cheese producing facilities in Austria at the day of production and after 14, 30, 90 and 160 days of ripening. i) Illumina MiSeq sequencing, ii) TaqMan quantitative real-time PCR (qPCR) and iii) cultivation approaches were used.

Results: i) The culture-independent results, obtained using high-throughput Illumina ITS2 (ITS3/ITS4) sequencing summarized the current knowledge on yeast and filamentous fungi in VB rinds. ii) The quantitative analysis (part 2) using 18S rRNA qPCR provided information about the quantitative level of fungi during ripening and revealed differences between the two ripening cellars. iii) Fungi culture collection (part 3) provided isolates to understand the importance of interactions between fungi and bacteria in cheese rinds. These new findings enable us to understand the VB cheese-making process better and might allow the processing- and ripening conditions to be improved to enhance the quality of the product.

PW406 Microbial diversity of Pélardon, an artisanal raw goat milk cheese

Marine Penland[1;2], Monika Coton[2], Jérôme Mounier[2], Sandrine Parayre[1], Emmanuel Coton[2], Hélène Falentin[1], Stéphanie Marie Deutsch[1]

[1]Institut National de la Recherche Agronomique, Science et Technologie du Lait et de l'Œuf - UMR1253, Rennes, France
[2]Université de Brest, Laboratoire Universitaire de Biodiversité et Ecologie Microbienne - EA3882, Plouzané, France

Background: Artisanal fermented foods constitute a significant part of the Mediterranean diet and represent a cultural heritage that needs to be preserved and protected; this is particularly true in the context of industrial food standardization. The manufacture and unique sensorial properties of such products mainly relies on autochthonous complex microbiota and their activities. These complex consortia are poorly characterized despite their major contribution to fermented food typicity.

Objectives: The present study focuses on Pélardon, a Protection Designation of Origin (PDO) French artisanal soft rind cheese, exclusively produced from raw goat's milk using a back slopping method in mountainous areas in southern France.

Methods: As part of the European project "ProMedFoods", aiming at promoting Mediterranean fermented foods through a better management of microbial resources, Pélardon microbial ecology and dynamics were studied by culture-dependent and -independent methods. A complete production was followed and sampling was performed at different cheese-making steps from the raw milk to cheese ripened for 3 months.

Results: Focusing on cultivable microorganisms, higher microbial populations were observed in whey used for inoculation compared to raw milk. Results also highlighted the importance of fungi. Despite the initial low inoculum, yeast growth began as early as milk acidification and during the renetting step. Differences in both fungal and bacterial communities were also observed between cheese rind and core samples. To better understand the origin of the observed microbial diversity, environmental and raw material samples were also investigated and ongoing work should provide new insights about the dynamics of these microbial communities.

PW407 Application of the LAMP method for food pathogen detection

Eva Svirakova[1], Madgalena Hruba[1], Katerina Loupancova[1]

[1]*UCT Prague, Department of Food Preservation, Czech Republic*

Background: The LAMP method ('loop-mediated isothermal amplification') is a modern method for rapid and conclusive detection of pathogenic or indicator microorganisms in various food matrices with considerable potential for the future. Its main advantages include speed, robustness, high specificity and selectivity of its reaction.

Objectives: The aim of this work is application of the LAMP method for fast and reliable detection of food pathogens in various food matrices purposefully contaminated under laboratory conditions.

Methods: The LAMP method was used for the experiment with 10 food matrices based on following methodology. Three samples were prepared from each matrix: one sample was control and two samples were contaminated by *Escherichia coli* O157:H7 ATCC 43888 and *Salmonella enterica* subsp. *enterica* ATCC 51741 (inoculum 10^2 CFU g^{-1} food matrix). For the back-detection of pathogens were used so-called the LAMP 'food panels' (the supplier: Amplex Biosystems GmbH, DEU).

Results: It was found that in 19 of 20 tested samples, intentionally contaminated by *Salmonella enterica* subsp. *enterica* ATCC 51741, was realized successful back detection from 95 % and that in 15 of 20 samples, intentionally contaminated by *Escherichia coli* O157: H7 ATCC 4388, was realized successful back detection from 75 %. The LAMP method can be used in the food industry at aimed protection of health safety of food raw materials and products. This work was supported by the Ministry of Agriculture of the Czech Republic, by the project QK1710156 (02/2017−12/2021, MZE/OK), in the programme ZEME (2017−2025).

PW408 Presence of non-typhoidal Salmonella adapted to diverse stresses in raw chicken meat, Portugal

Sofia Ribeiro[1], Andreia Rebelo[1;2], Joana Vanessa Cordeiro Melro Mourão[1], Carla Novais[1], Luisa Maria Vieira Peixe[1], Patricia Antunes[1;3]

[1]*UCIBIO/REQUIMTE, Microbiology Laboratory, Faculty of Pharmacy, University of Porto, Portugal*
[2]*School of Health, Polytechnic Institute of Porto, Portugal*
[3]*Faculty of Nutrition and Food Science, University of Porto, Porto, Portugal*

Background: *Salmonella* causes frequently foodborne infections mainly associated with eggs/poultry products. A decreasing trend in human salmonellosis, particularly of *S*.Enteritidis, has been observed in EU due to successful control programs at the avian production level. However, the effectiveness of control practices on the elimination of *Salmonella*, and particularly of EU targeted serotypes, in poultry has been scarcely explored

Objectives: Here, we investigated the presence of *Salmonella*, and their clinically-relevant serotypes, among fresh chicken-meat samples at final processing level.

Methods: Pooled chicken-meat samples (n=53; each sample=25g of neck skin from 10 carcasses; 29 producers) were collected in 2018 (spring/summer). Samples were processed following ISO-6579-1:2017 cultural standard and a molecular approach (PCR-*invA* gene) applied directly in the pre-enrichment/enrichments broths. Search of EU targeted serotypes (Enteritidis/Typhimurium/4,5,12:i:-) and their antibiotic/metal resistance markers were performed by PCR. Ability to survive/grow at acidic pH (2-6.5) was evaluated by broth-microdilution.

Results: *Salmonella* was detected in 4%-two samples of fresh chicken-meat from different poultry-farms/both seasons (cultural+molecular approaches). The isolates belonged to a non-H_2S-producing serotype *S*.4,5,12:i:- (n=6 isolates/spring sample), with the typical antibiotic/metal resistance genotypic features of clones currently circulating in Europe, or *S*.Enteritidis (n=3 isolates/summer sample). Isolates grew at minimal pH=4-4.5 and survived until pH=3.5-4. A low occurrence of *Salmonella* in chicken carcasses was observed, although with detection of two serotypes of public health significance with ability to grow under diverse stresses. Our results alert for the need to evaluate current biosafety measures to prevent the spread of these pathogens in the poultry production through the consumer.

PW409 Interaction between Campylobacter jejuni and Bacillus subtilis during biofilm formation

Andi Erega[1], Katarina Šimunović [1], Polonca Stefanic[1], Anja Klančnik[1], Iztok Dogsa[1], Ines Mandic-Mulec[1], Sonja Smole-Možina[1]

[1]Jamnikarjeva101, University of Ljubljana, Biotechnical Faculty, Department of Food Science and Technology, Ljubljana, Slovenia

Background: Biofilms represent protective environment and improve survival of enteric pathogens transmitted along the food chain. *Campylobacter jejuni* is physiologically unique foodborne pathogen and can be sheltered in multispecies communities. Bacillus species are probiotics that successfully decrease *C. jejuni* colonization, however mechanisms that contribute to competitive exclusion of the pathogen are still poorly understood.

Objectives: The aim of the study is to investigate the mechanisms behind competitive exclusion of *C. jejuni* by *Bacillus subtilis* natural isolates during biofilm formation.

Methods: We co-cultured wild type of *B. subtilis* and *C. jejuni* or mutants of each species with the wild type competitor in static Mueller-Hinton broth. Fitness (W) and adhesion rate of each species was determined by CFU. Dynamics of biofilm formation in co or monoculture was documented by photography and by confocal laser scanning microscopy.

Results: *B. subtilis* natural isolate during co-culture with *C. jejuni* prevent *C. jejuni* biofilm formation and decreased the fitness of *C. jejuni* by 3,4 log10 in static co-culture, while adhesion on abiotic surface was decreased by 2,8 log10. *C. jejuni* mutants in genes encoding an efflux pump were more susceptible to *B. subtilis* attack, suggesting that interference competition play a role in outcome competition between two species. Oxygen potentially modulates *C. jejuni* exclusion in co-culture with *B. subtilis*. Biofilm formation may contribute to *B. subtilis* competitiveness. The *B. subtilis* cell free supernatant increased formation and thickness of *C. jejuni* biofilm during the first 15h of incubation, while adhesion on abiotic surface was decreased.

PW410 Effect of cranberry (Vaccinium macrocarpon) powder on fecal microbiota of Wistar rats

Mayot Gilles[1], Chettaoui Rayane[1], Patrick Di Martino[1]

[1]*Université de Cergy-Pontoise, Laboratoire ERRMECe*

Background: Cranberry (*Vaccinium macrocarpon*) dietary supplementation can help prevention of urinary tract infections. This cranberry activity is related to its particular content of proanthocyanidin-type polyphenols (PACs).

Objectives: The present study was done to investigate whether intake of cranberry powder affects concentrations of thermotolerant coliforms, *Enterococcus* spp. and *Lactobacillus* spp. in rat feces.

Methods: The study was a randomized and cross-over experiment. Twelve rats, housed in individual cages, received successively two diets as pellets during 7 days each: a standard diet without polyphenols and the standard diet supplemented with cranberry powder containing 10.9 mg/100g of PACs. There was a 7 days wash-out period in between with standard diet without cranberry. Body weight and feed intake were recorded regularly. Feces were collected on the last day of treatment, and crushed to count the different bacterial populations using the most probable number method. Thermotolerant coliforms were grown in BGBLB tubes and on MacConkey agar plates. *Enterococci* were grown in Rothe broth, Litsky broth and on Streptococcal Kenner fecal agar plates. *Lactobacilli* were grown in Man Rogosa Sharpe broth.

Results: Body mass gains were not affected by cranberry supplementation. This is consistent with equal food intake, cranberry powder not providing significant energy supplement. Cranberry powder supplementation was associated with changes in fecal concentrations of thermotolerant coliforms, *Enterococci* and *Lactobacilli* in some rats, but did not induce significant changes in these concentrations in a global population of 12 rats.

PW411 A web based genotyping pipeline program for foodborne pathogen genomic epidemiology

Sojin Ahn[1], Soyun Jhang[1], Heebal Kim[1;2]

[1]Seoul National University, Interdisciplinary Program in Bioinformatic, Seoul, Korea, Rep. of South
[2]Seoul National University, Department of Agricultural Biotechnology and Research Institute of Agriculture and Life Sciences, Seoul, Korea, Rep. of South

Background: Conventional typing methods have been verified by many researchers, but nevertheless have been replaced by methods using whole genome sequencing, due to the clear limitations. For example, the SNP-based comparative genomic and phylogenetic methods are widley used in foodborne outbreak investigation. However, for those who are not familiar with bioinformatics still find it difficult to use Linux-based programs and it is important for the program to perform better. Therefore, we developed an accurate and fast web-based epidemiological typing program

Objectives: In this study, we developed an epidemiological typing program that not only compares the already known foodborne pathogens, but also allows easy access to process an accurate analysis for people with no prior knowledge on bioinformatics.

Methods: First of all, we developed an pipeline that can quickely and accurately validate the foodborne pathogens. In order for the benchmarking test of the pipeline, the accuracy and analysis time were calculated using simullation data. Moreover, the comparisons with the PFGE method was assessed by using the actual PFGE pattern and the public database of food poisoning outbreak information.

Furthermore, we have already created database which we analyzed more than 2,000 data using our pipeline program to compare the core SNP and the existing public database of Foodborne pathogens.

Results: As a result, we have developed a more accurate and applicable pipeline program than the existing typing program. We expect that it could be possible to rapidly to the food poisoning outbreaks by applying our newly developed program.

PW412 UV-C tolerance of microorganisms from RO treated dairy water streams

Susanne Knøchel[1], Eirini Vitzilaiou[1]

[1]*University of Copenhagen, Food Science, Denmark*

Background: Ultraviolet (UV) irradiation is commonly used for process water treatment since it is highly effective against a wide spectrum of microorganisms and an easy-to-use technology. Several food processing industries are starting to apply Reverse Osmosis (RO) membrane filtration and UV-C treatment to recondition process water for re-use. Although RO membranes in principle remove all microorganisms, some microorganisms may form biofilm on permeate membrane surfaces.

Objectives: The UV-C tolerance of microorganisms isolated from whey water permeate streams and RO membrane surfaces was investigated and compared to that of pathogenic bacteria.

Methods: The UV-LED device PearlBeam™ (Aquisense Technologies, USA) was used in a lab-scale set-up. Different UV-doses at 255nm were applied to the filamentous yeast species *Saprochaete clavata* and *Magnusiomyces spicifer*, and the gram-negative bacteria *Klebsiella sp., Raoultella sp.,* and *Pseudomonas fluorescens.* A heat resistant *Salmonella* isolate and a methicillin resistant *Staphylococcus aureus (*MRSA) strain were also tested.

Results: The filamentous yeast showed significantly higher resistance than bacteria, decreasing only 1-2 $\log_{10}$ after a UV-dose of 10 mJ/cm^2 compared with decreases of 4-5-$\log_{10}$ for bacteria. The yeast strains needed doses 4-6 times higher to reach similar reductions. This enhanced tolerance underlines that non-bacterial microorganisms may be important to consider in biofouling interventions.

PW413 Identification of pyridoxal phosphate dependent enzymes from Pediococcus acidilactici possibly involved in the amino acid metabolism of cheese

Alexander Wenger[1], Weisskopf Laure[1], Eugster Elisabeth[2], Bachmann Hans-Peter[3], Irmler Stefan[3]

[1]*University of Fribourg, Department of Biology, Fribourg, Switzerland*
[2]*Berner Fachhochschule – Hochschule für Agrar-, Forst- und Lebensmittelwissenschaften HAFL, Zollikofen, Switzerland*
[3]*Agroscope (Liebefeld), Liebefeld, Switzerland*

Background: *Pediococcus* ssp. are lactic acid bacteria that frequently occur in fermented foods and beverages. In cheese, their role on ripening and flavor development is not well understood. The species *Pediococcus acidilactici* is of special interest because it degrades threonine and serine and concomitantly forms alanine and α-aminobutyrate. We assume that pyridoxal phosphate (PLP) dependent enzymes are involved in the metabolism of these amino acids.

Objectives: Identification of PLP-dependent enzymes from *P. acidilactici* strain FAM18098 that are involved in threonine and serine catabolism.

Methods: The whole genome of *P.acidilactici* strain FAM18098 was sequenced using long-read sequencing technology. The assembled and annotated genome was searched for genes encoding PLP-dependent enzymes. Genes of interest were cloned and heterologously expressed in *Escherichia coli.* The purified proteins were characterized using photometric assays, which detected aminotransferase- and cystathionine lyase activity. Additionally, HPLC analysis was performed to detect and identify the release of organic acids.

Results: Two genes encoding enzymes with PLP-dependent type-I domains were cloned from *P. acidilactici* FAM18098. One of the recombinant proteins catalyzed the transamination of methionine, branched chain-amino acids, phenylalanine, and α-aminobutyrate using α-ketoglutarate as cosubstrate. The pH optimum of this reaction was at 7.4. The other recombinant protein was found to be a cystathionine β-lyase that degraded L-cystathionine to L-homocysteine and pyruvate. The pH optimum of this reaction was at 9.2. Both enzymes showed low activity at pH 5.5 , a pH which is usually present in cheese.

PW414 Antimicrobial resistance profiles of foodborne pahogens associated with vemicompost and vermicompost tea

Abimbola Atanda[1], Rasheed Adeleke[2], Evelyn Madoroba[3], Piet Jooste[4]

[1]*Agricultural Research Council, South Africa*
[2]*North-West University, Potchefsroom, South Africa*
[3]*University of Zululand, South Africa*
[4]*Tshwane Unversity of Technology, South Africa*

Background: Raw materials used in the production of vermicompost (VC) and vermicompost tea (VCT) may harbour various pathogenic bacteria responsible for a number of animal and human diseases worldwide. The identification and characterisation of such pathogenic organisms is necessary for assessing the safety of these products.

Objectives: In the present study, our goal was to determine the presence of possible pathogens in VC and VCT and, if present, to determine the antimicrobial susceptibility profiles of the organisms. VC and VCT samples were collected from five different farms in the Winterveldt, South Africa.

Methods: Vermicompost and VCT samples were collected from five different farms in the Winterveldt, Pretoria, South Africa. A combination of both culture dependent and molecular techniques was used to analyse the samples.

Results: Only one out of 60 VC and VCT samples was found to contain a potentially pathogenic organism. The use of phenotypic procedures aided the final identification of the isolate to be confirmed as Salmonella enterica serovar Typhimurium. It tested positive for species specific invA genes. Antibiotic testing using the agar diffusion technique showed that the Salmonella isolate was resistant to only kanamycin. The Salmonella counts that were observed in this study were lower than the general infective doses of these bacteria. In light of these findings, it was concluded that the VC and VCT produced by these farmers presented a low risk in terms of the safety of the products.

PW415 The definition of genetic determinants of pathogenecity in bacteria of genus Campylobacter from different origions

Dmytro Kyryk[1], Denis Nechaiev[1]

[1]Shupyk National Medical Academy of Postgraduate Education, Department of microbiology and epidemiology, Kyiv, Ukraine

Background: The definition of genetic determinants of pathogenicity(GDP) in strains of different origins is an important way of epidemiological studies.

Objectives: The aim of the study was to evaluate the epidemic risk of poultry products(PP)on the basis of determining the GDP of Campylobacter strains from different origin.

Methods: . To investigate the GDP were selected 97 strains of Campylobacter from PP and from 48 patients with acute diarrhea(PAD). To identify determinants of citolethality (CLT)and invasiveness(IV) were selected primers from databases of GeneBank, EMBL, DDBJ and Entrez.The tests were carried out in native amplifier in 25 µl volumes for 3,5 h and analyzed by electrophoresis test .

Results: The strains of C. jejuni isolated from PAD and PP were studied the pathogenicity genes responsible for producing CLT: CdtA, CdtB, CdtC and IV: VirB8, VirB9, and VirB11. The diarrhea syndrome is only possible in the combined action of the three subunits of CLT. In all strains isolated from PP were identified two genes of CLT - CdtA, CdtC. However, all three subunits of CLT were frequently detected in samples of chilled meat – 68.6 % of strains..Campylobacter strains isolated from PAD established genes CdtA and CdtC in 100% and CdtB gene in 73 %. Genetic determinants of IV – VirB8 and VirB11 were also identified only in clinical strains and strains isolated from samples of chilled meat.The similarity of GDP of Campylobacter strains, isolated from PAD and PP, confirms the presence of the relationship of epidemic and epizootic processes.

PW416 Extending shelf-life of a cereal-based infant food through fermentation with anti-fungal Lactobacilli

Celia Álvarez[1], Patricia Ruas-Madiedo[1], Ana Rodríguez[1], Beatriz Martinez[1]

[1]*Instituto de Productos Lácteos de Asturias IPLA - CSIC, Villaviciosa, Spain*

Background: Cereal-based foods are the main source of nutrients for malnourished children in developing countries. However, cereals are frequently contaminated with fungi, representing a serious safety concern due to the production of mycotoxins. Fermentation by selected lactic acid bacteria has a long history of safe use and can be regarded as an effective way to reduce fungal contamination.

Objectives: Our goal was to assess the ability of a collection of *Lactobacillus* sp. to grow on a cereal-based food matrix and antagonize fungal development during fermentation and storage at low temperature.

Methods: Fermentation experiments were carried out at 30 °C in a nutritional formula recommended by FAO composed of corn flour, skim milk powder and soy flour (60:25:10) diluted at 15% in water. Contamination experiments were carried out with 4 different fungal species by adding spores before or after fermentation and storage at 6 °C.

Results: All the *Lactobacillus* strains were able to grow in the nutritional formula but differences on the final pH and lactic acid concentration were observed. When the nutritional formula was contaminated with fungal spores before fermentation, *Aspergillus oryzae* and *Aspergillus parasiticus* were inhibited for 9 days and 4 days, respectively. *Penicillium expansum* and *Gibberella moniliformis* developed as fast as in the unfermented controls (3 d). In post-contamination experiments, only *P. expasum* and *G. monoliformis* were able to grow at 6 °C. None of the lactobacilli inhibited *P. expasum* but 3 of them reduced by 3 log units the growth of *G. monoliformis.*

PW417 In vitro antilisterial effect of essential oils by modeling of growth curve MIC values

Biljana Nikolić[1], Bojana Vasilijević[1], Marina Jovanović[1], Jelena Knežević-Vukčević[1], Ilija Djekic[2], Stefana Cvetkovic[1], Stefana Djukanovic[1], Dragana Mitić-Ćulafić[1]

[1]University of Belgrade - Faculty of Biology, Chair of Microbiology, Belgrade, Serbia
[2]University of Belgrade - Faculty of Agriculture, Department of Food Safety and Quality Management, Belgrade, Serbia

Background: *Listeria monocytogenes* is food contaminant being widely tolerant to different environmental factors. It is one of the most frequent causative agents of deaths among foodborne pathogens.

Objectives: The aim was to examine the *in vitro* antilisterial effect of essential oils (EOs) of *Juniperus communis* and *Satureja montana*.

Methods: EOs were chemically analyzed by GC/GC-MS. Antilisterial effect against ATCC19111 strain and three primoisolates was determined in microdilution, checkerboard and time-kill assays. Results of time-kill assay were used not only to analyze the type of interaction between EOs, but also to determine the growth rates of curves obtained for different concentrations of test substances. By plotting of growth rates as a function of applied concentrations, growth curve MIC was determined as the concentration inducing neither the progression nor the regression of bacterial population.

Results: MIC values determined in microdilution assay and by modeling of time-kill assay growth curves were in the ranges 0.5-1% and 0.03-0.14%, respectively. Multifold higher values of MIC obtained in microdilution assay could be addressed to the feature that *L. monocytogenes* is more resistant to different stressors in reduced oxygen environment. In addition, synergism of both EOs in mixture treatment of isolates was determined in checkerboard and time-kill assays. In conclusion, *J. communis* and *S. montana* EOs and their combinations could be the efficient inhibitors of the *L. monocytogenes* growth. However, the fact that its susceptibility to antimicrobials is influenced with environmental conditions requires the use of different experimental approaches in order to better explore it.

PW418 Characterization of antibacterial peptide isolated from Staphylococcus equorum KS1039, the dominant strain of fermented fish sauce

Eunhye Jo[1], Jihye Kim[1], Areum Lee[1], Jaeho Cha[1]

[1]*Pusan National University, Microbiology, Busan, Korea, Rep. of South*

Background: *Staphylococcus equorum* KS1039 has been identified as the dominant species of Saeu-jeotgal, a high salt-fermented seafood of Korea. KS1039 was sensitive to various antibiotics, and was negative for hemolytic activity, biofilm formation, and biogenic amine production. The strain exhibited protease and lipase activities for enhancement of sensory properties. It exhibited a bacteriostatic effect on gram-positive bacteria including *Bacillus cereus and Listeria monocytogenes*, whereas other pathogens including Gram negative bacteria were not susceptible.

Objectives: We isolated the antimicrobial peptide from *S. equorum* KS1039 and examined the action mechanism as well as the antimicrobial properties of this peptide. Additionally, the utility of strain KS1039 as a starter for high-salt food fermentation was examined.

Methods: The antibacterial peptide was purified using hydrophobic, ion-exchange, and size exclusion chromatography. Its antibacterial activities against several bacteria and the stability toward pH, temperature as well as proteases were measured by disc diffusion assay. The identification of the peptide was analyzed by mass spectrometry and peptide sequencing.

Results: The production of antimicrobial peptide was the highest at the early stationary phase. The estimated molecular mass of the purified peptide was approximately 6,000 Da. The peptide was stable on the wide pH range of 2-9 and heat. Moreover, it was not degraded by proteases unlike other antibacterial peptides. The unique characteristics of this peptide suggests that it is a novel class II-type bacteriocin. It is a first report of the antibacterial peptide from *S. equorum*. KS1039 strain has a potential as a starter culture for fish fermentation.

PW419 Comparative Pan-genome Analysis-Based Serotype Determination of Salmonella enterica subsp. enterica Using Real-Time PCR

Joon-Gi Kwon[1], Sang Ho Choi[2], Hyeun Bum Kim[3], Ju-Hoon Lee[1]

[1]*Kyung Hee University, Food Science and Biotechnology, Yongin, Korea, Rep. of South*
[2]*Seoul National University, Food and Animal Biotechnology, Korea, Rep. of South*
[3]*Dankook University, Animal Resources Science, Cheonan, Korea, Rep. of South*

Background: Pathogenic *Salmonella enterica* are the most common food-borne pathogens over the world, causing salmonellosis. This species is composed of more than 2,600 serotypes and the identification is very important in epidemiological studies.

Objectives: However, the conventional serological method is expensive, laborious, and time-consuming. Therefore, it is necessary to develop a new simple method to identify the serotype of *S. enterica*

Methods: In this study, comparative pan-genome analysis of *S. enterica* showed the presence of serotype-specific genes to differentiate *S*. Typhimurium (*hxlA*) and *S*. Enteritidis (*hokC*) from other serotypes of *S*. *enterica*, suggesting the possibility of Real-Time (RT) PCR approach for their serotype identifications

Results: To validate this, 18 strains from seven different serotypes of *S. enterica* were selected. In addition, based on the serotype-specific gene sequences, primer-probe sets targeting these specific genes were designed to develop RT-PCR method. Comparative RT-PCR results confirmed that this method works perfectly for identification of serotypes of *S*. Typhimurium and *S*. Enteritidis from other serotypes of *S. enterica*. This RT-PCR method would be useful for their rapid detection, differentiation, and identification. Furthermore, this method may be extended to detect, differentiate, and identify various serotypes of *S. enterica* via more experiments in near future. This rapid detection and identification method may contribute to food safety from food-borne outbreaks via ingestion of various *Salmonella*-contaminated foods.

PW420 Staphylococcus aureus biofilm producers colonizing employeers of "Piloto Kitchens" of Brazil

Rafael Silva Rosa[1], Andre Aparecido Dias[1], Diego Junior Santos Gonçalvez[1], Camila Jesus Silva[1], Giovana Nascimento Pereira[1], Thais Batista Carvalho[1], Valeria Cataneli Pereira[1]

[1]*University of West Paulista, Microbiology, Presidente Prudente, Brazil*

Background: Maintained by the National School Feeding Program of Brazil, “Pilot Kitchens” play an important role in school feeding, since they are responsible for the elaboration and distribution of the meal in public schools, serving children of adolescents. However, these foods are subject to bacterial contamination due to hygiene failure of food handlers.

Objectives: This study aimed to evaluate the colonization by *S. aureus* in employees of “Pilot Kitchen” and to determine the biofilm production capacity of these bacteria.

Methods: Nasal and hand samples from 27 professional cooks were collected through swab and *S. aureus* were identified by PCR. *S. aureus* were submitted to adhesion tests in borosilicate and red congo agar (CRA) tubes to verify biofilm production and the *icaA* and *icaD* genes were detected by PCR.

Results: Of the 54 samples collected, 92.6% were *S. aureus*. Biofilm production was detected in 92% of *S. aureus* by CRA method and 94% by adhesion method. The sensitivity of the PCR technique to the *icaA* and *icaD* gene was 92% and 94%, respectively. The adhesion method in borosilicate tubes presented 90% and 94% sensitivity with the *icaA* and *icaD* genes, respectively. There was no specificity and agreement between the techniques. The data indicate that all the employees were colonized by *S. aureus* with high capacity of biofilm formation, emphasizing the importance of biosafety norms in these places.

FAPESP (2018 / 00056-0)

PW421 Identification and characterization of amylolytic lactic acid bacteria (LAB) isolated from traditional Bulgarian sourdoughs by Biolog system and PCR screening of the genes encoding amylolytic activity

Mariana Petkova[1], Petya Stefanova[2], Velitchka Gotcheva[2], Iordanka Kuzmanova[1], Angel Angelov[2]

[1]Agricultural University of Plovdiv, Microbiology and Environmental Biotechnology, Plovdiv, Bulgaria
[2]University of Food Technology, Biotechnology, Plovdiv, Bulgaria

Background: The main phenotypic identification characteristics of amylolytic LAB is carbohydrate utilization. It is generally accepted that most of the lactic acid bacteria utilize few carbohydrates. Biolog microstation system has been used for biochemical screening and identification. Genetic analysis of starch utilizing strains of LAB has revealed the existence of six genes, involved in the expression of the amylolytic capability.

Objectives: The aim of the project is to investigate the basic molecular and biochemical characteristics of amylolytic lactic acid bacteria isolated from 20 traditional Bulgarian sourdoughs.

Methods: LAB strains were selected based on their amylolytic activity. Identification of the bacteria was performed using by Biolog system. The presence in the chromosome of the 6 genes, involve of amylolytic activity was determined by classical PCR methods.

Results: Biolog system revealed presence of *L. lactis*, *L. mesenteroides*, *L. plantarum*, *L. brevis, L. curvatus* and *L. sakei* with 90% probability. When comparing the biochemical profile of the tested microorganisms was found that all of them absorb typical lactic acid bacteria carbon sources: dextrin, D - fructose, α - D - glucose, maltose, maltotriose, D - mannose, 3 - methyl - D - glucose, palatinose, L-lactic acid, D-lactic acid. The results from PCR analysis from the presents of amylolytic genes showed that 97% of the isolates have the *glgP* gene, the 90.1% *dexC* gene, the 94.2% *α-amy* gene, the 97% - *ag1* gene, and 97% *malP* and 91.5% *malL* genes. Thirty-five strains possess high amylolytic activity, determined by the presence of tested amylolytic genes.

PW422 Microbial diversity of naturally fermented PDO Nyons table olives unraveled

Marine Penland[1;2], Stéphanie Marie Deutsch[2], Hélène Falentin[2], Christophe Le Meur[1], Emmanuel Coton[1], Jérôme Mounier[1], Monika Coton[1]

[1]*Université de Brest, Laboratoire Universitaire de Biodiversité et Ecologie Microbienne - EA3882, Plouzané, France*
[2]*Institut National de la Recherche Agronomique, Science et Technologie du Lait et de l'Œuf - UMR1253, Rennes, France*

Background: Artisanal fermented foods constitute a significant part of the Mediterranean diet and represent a cultural heritage that needs to be preserved and protected. PDO Nyons table olives are produced according to a traditional process by a slow spontaneous fermentation in brine. The manufacture and unique sensorial properties of these olives thus only rely on autochthonous complex microbiota.

Objectives: This study aimed at unraveling the microbial ecology and dynamics of Nyons table olives during yearlong spontaneous fermentation and establishing the main involved microorganisms.

Methods: Fermentations were monitored at a local producer plant at regular intervals for two harvest periods and for two olive types (organically and conventionally cultivated olives) through culture-dependent and metagenetic (targeting ITS2 for fungi and V3-V4 region for bacteria) approaches. Brine and olives were also sampled for volatile and biochemical parameters.

Results: Total microbial populations on fresh fruits were low around 3 log CFU/g. Acidification occurred during the first 21 days of fermentation although lactic acid bacteria populations were scarce. Yeasts were the most dominant population during the entire fermentation. Interestingly, bacterial communities were dominated by the *Celerinatantimonas* genus in both brine and olives while lactic acid bacteria and *Enterobateriaceae* were sub-dominant, confirming culture-dependent results. ITS2 sequencing data revealed a complex fungal diversity dominated by *Citeromyces*, *Wickeramomyces*, *Saccharomyces* and *Pichia* genera; the latter two genera being at higher counts in olive samples than in brine. The obtained data is currently being used to select key microorganisms of technological interest for pilot scale experiments to improve fermentation control.

PW423 The combined effect of gamma irradiation and modified atmosphere packaging on microbial decontamination of turmeric powder

Saeideh Esameili[1], Amir hossein shahali[2]

[1]National Nutrition and Food Technology Research Institute, Shahid Beheshti University of Medical Sciences, Department of Food Technology Research, tehran, Iran
[2]Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Department of Health and Community Medicine, tehran, Iran

Background: Poor hygienic conditions of harvesting, processing, storage and handling of spices caused them potent for microbial contaminations also, Conventional methods are not appropriate for decontamination of them. Modified atmosphere packaging by restricting microbial growth and reducing destruction of food components could be effective to reduce undesirable effects of gamma irradiation.

Objectives: This study evaluated the combined effects of gamma irradition under various atmospheres of packaging (vcuum, nitrogen and air) on microbial decontamination of turmeric powder.

Methods: Turmeric rhizomes powder packaged under various atmosphere (vacuum, N_2 and air) using high-barrier multilayered film. Then, gamma irradiated at 5, 10, and 15 kGy doses. Microbial contamination of samples evaluated by total aerobic bacterial count, spore forming bacterial count, total yeast/mold count and coliforms count.

Results: The results showed the effectiveness of gamma irradiation on microbial decontamination of the samples. The microbial contamination was about 10^7 cfu/g for total aerobic bacteria and spore forming bacteria and 10^4 cfu/g for coliforms and yeasts and molds at initial and then by irradiation at the dose of 5 kGy reduced nearby 3, 3, 4, and 4 log cfu/g, respectively. By increasing irradiation dose from 5 to 15 kGy, microbial counts were not detectable at the highest dose. The combined effect of gamma irradiation and packaging atmosphere on decontamination of samples was not significant. The minimum irradiation dose required for ensuring the microbial safety of the samples is 8.28 kGy, which is lower than 10 kGy, the approved safe irradiation dose for aromatic spices.

PW424 Influence of shaking and viable cell numbers on microbial conjugated linoleic acid (CLA) production

Ana Luiza Fontes[1;2], Lígia Pimentel[1;2;3], Ana Sofia Salsinha[1], Juan Miguel Rodriguez[4], MR Domingues[5;6], Luís M. Rodríguez-Alcalá[1], Ana Maria Gomes[1]

[1]*Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia*
[2]*QOPNA – Unidade de Investigação de Química Orgânica, Produtos Naturais e Agroalimentares, Universidade de Aveiro*
[3]*CINTESIS – Centro de Investigação em Tecnologias e Sistemas de Informação em Saúde, Faculdade de Medicina da Universidade do Porto*
[4]*Departamento de Nutrición, Bromatología y Tecnología de los Alimentos, Facultad de Veterinaria, Universidad Complutense de Madrid*
[5]*Mass Spectrometry Centre, Department of Chemistry & QOPNA, Universidade de Aveiro, Campus Universitário de Santiago*
[6]*Department of Chemistry & CESAM & ECOMARE, Universidade de Aveiro, Campus Universitário de Santiago*

Background: Conjugated linoleic acid (CLA) isomers are naturally produced from dietary linoleic acid (LA) by ruminal bacteria, but several probiotic strains have also revealed the capacity to produce these bioactive compounds. The amount of CLA produced is strain-dependent and may also be influenced by cultivation conditions.

Objectives: *In vitro* studies usually test CLA production by growing strains in culture medium containing a specific LA concentration, but few have applied shaking during incubation; shaking may improve access to substrate. Hence, this work aimed to study the influence of shaking on CLA production.

Methods: *Bifidobacterium breve* DSM 20091*, Lactobacillus gasseri* VR-III-51, *L. plantarum* DSM 20205 and *L. sakei* 20017, previously identified as CLA-producers, were selected for this study. They were inoculated (1% v/v) in MRS medium, containing 0.5 mg/mL of LA and incubated at 37 °C for either 24, 48 or 72 h, with and without shaking (150 rpm). Viable cell numbers were determined and fatty acid concentration was analyzed by gas chromatography.

Results: Shaking reduced LA conversion rates for all strains except *L. gasseri* VR-III-51. In what concerns correlation between viable cell numbers at the end of each incubation period and LA conversion rates, important differences were observed: a negative correlation for *L. gasseri* VR-III-51 with shaking, a positive correlation for *B. breve* DSM 20091 without shaking and no correlation for *L. plantarum* 20205 and *L. sakei* DSM 20017, with/without shaking were observed. In conclusion, shaking and viable cell numbers (incubation time) affect microbial CLA production in a strain-dependent manner.

PW425 Metagenomic analysis reveals the key microbes and metabolic features during fermentation of ganjang, Korean traditional soy sauce

Byung Hee Chun[1], Che Ok Jeon[1]

[1]*Chung-Ang University, Life Science, Seoul, Korea, Rep. of South*

Background: Ganjang is the Korean traditional fermented soy sauce. Research on the metabolic features of ganjang microbes during ganjang fermentation is important to understand the fermentation process of ganjang. However, most previous studies were limited to a analysis of either the microbial communities or the metabolites during ganjang fermentation.

Objectives: The aim of this work is to investigate the key microbes and their metabolic features during ganjang fermentation using metagenome analysis.

Methods: The community analysis using amplicon-based and raw metagenome sequencing data were processed and classified using Qiilme II and kaiju, respectively. Assembly and binning process of ganjang metagenome data were conducted using SPAdes and CONCOCT, respectively. The quality of binned genomes were assessed using CheckM program. The metabolic features of binned genomes were analyzed based on KEGG annotation.

Results: The community analysis using amplicon-based and raw metagenome sequencing data revealed that *Tetragenococcus*, *Chromohalobacter*, *Marinobacter*, *Halomonas*, *Idiomarina*, *Bacillus*, and *Staphylococcus*, *Aspergillus*, *Wickerhamomuces*, and *Debaryomyces* showed predominant during ganjang fermentation. The total 17 bacterial genomes were reconstructed through assembly and binning process. Additionally, two fungal strains were isolated and their genomes were sequenced for further genome study. The KEGG analysis showed that *Debaryomyces* and *Wickerhamomyces* harbor high number of genes related to lipid metabolism and genome analysis also showed that they harbor genes to metabolize diverse soybean lipids such as triacyl glycerol and lecithin. The genome analysis also showed that *Bacillus* and *Enterococcus* harbor genes related to metabolize pectin and *Bacillus, Tetragenococcus, Debaryomyces* and *Wickerhamomyces* harbor genes to metabolize starch.

PW426 Enterotoxin gene profiles of Staphylococcus aureus isolated from different food of animal origin processing plants

Weronika Korpysa-Dzirba[1], Jolanta Grażyna Rola[1]

[1]*National Veterinary Research Institute, Department of Hygiene of Food of Animal Origin, Puławy, Poland*

Background: The growth of *S. aureus* and potential production of staphylococcal enterotoxins (SE's) during manufacturing of food represents an important threat to the consumers health.

Objectives: The aim of this study was to estimate the relationship between staphylococcal gene profiles of *S. aureus* isolated from production plants of food of animal origin.

Methods: A total of 800 samples have been collected during a 5 year study in production lines of different types of food of animal origin in small-scale cow, goat and sheep dairy farms and food production plants. The collected samples included raw, half and the final products as well as swabs from the environment and hands of people involved in the process. Samples have been analysed according to ISO 6888-2 Standard. Detection of SE genes was done using multiplex PCRs.

Results: The contamination by coagulase-positive staphylococci was found in samples collected in all types of production lines with the prevalence between 9.9% in fish plants and 85,1% in sheep dairy farms. Over 50% of isolated CPS were enterotoxigenic with the exception of strains originated from sheep dairy farms where 12.6% had enterotoxin genes. The majority of enterotoxigenic strains had a combination of *seg* and *sei* genes alone (21.7%) or with *sea* (4.7%), *seh* or *sep* markers (2.8% each). These enterotoxin profiles were found in *S. aureus* from cow and goat dairy farms and in all types of food of animal origin plants. A significant percent of *S. aureus* had single *sec* (22.6%), *sep* (20.8%) or *seh* (14.2%) genes.

PW427 Antibacterial potential of red-wine marinades containing essential oils against food contaminants in raw beef

Dragana Mitić-Ćulafić[1], Bojana Vasilijević[1], Marina Jovanović[1], Jelena Knežević-Vukčević[2], Ilija Djekic[3], Stefana Cvetkovic[4], Stefana Djukanovic[4], Biljana Nikolić[4]

[1]*University of Belgrade-Faculty of Biology, Chair of Microbilogy, Belgrade, Serbia*
[2]*University of Belgrade-Faculty of Biology, Chair of Microbilogy, Belgrade,*
[3]*University of Belgrade-Faculty of Agriculture, Department of Food Safety and Quality Management, Belgrade-Zemun, Serbia*
[1]*University of Belgrade-Faculty of Biology, Chair of Microbilogy, Belgrade, Serbia*

Background: Marinades could be prepared with different constituents including wines and essential oils (EOs), using as flavoring agents and natural preservatives.

Objectives: The aim of this work was to monitor the antibacterial effect of red-wine marinades containing EOs of *Juniperus communis* (J), *Satureja montana* (S) and their mixtures (J+S) on beef.

Methods: Marinades - the base one (BM), and the ones with J, S and J+S were prepared, while saline (0.85% NaCl) was used as negative control. Samples were marinated for 24h at 4°C and used for sensory and microbiological analyses. The taste and odor were rated by panelists using the intensity scales. The bacterial growth of aerobic heterotrophic mesophils (AHM), Enterobacteriaceae, lactic acid bacteria, and inoculated *Listeria monocytogenes*, was monitored in time-kill assay performed during 15 days of meat storage at 4°C.

Results: Sensory acceptable concentrations were up to 0.5%, 0.125% and 0.25%+0.125% for J, S and J+S, respectively. They were used in time-kill assay which revealed that bacterial counts of all monitored groups were significantly dropped in the marinades, comparing to saline. The remarkable effect was achieved with BM, with the drop of approximately 2-3 logCFU/g. The addition of EOs improved the antibacterial effect and additionally decreased the logCFU/g for 1-2.5 units. The further enhance of antibacterial efficacy with EOs mixture was observed in the cases of *L. monocytogenes* ATCC191111 and AHM. This work elucidated that *S. montana* and *J. communis* EOs could provide a safety barrier against *L. monocytogenes* and food spoilage bacteria and encouraged further research.

PW428 Antimicrobial resistance of Yersinia enterocolitica isolated from raw goat milk

Anna Czubkowska[1], Jolanta Grażyna Rola[1]

[1]*National Veterinary Research Institute, Department of Hygiene of Food of Animal Origin, Puławy, Poland*

Background: *Yersinia enterocolitica* is an important food-borne pathogen responsible for yersiniosis. Therefore, the monitoring of these bacteria's susceptibility to antimicrobial substances is very important, as it can be the basis for choosing siutable therapeutic procedures in *Y. enterocolitica* infections.

Objectives: The aim of the study was to evaluate the antimicrobial resistance of *Y. enterocolitica* isolated from raw goat milk on dairy farms in Poland.

Methods: In total, 48 strains of *Y. enterocolitica* isolated in the years 2016-2017 from raw goat milk were selected for this study. Antimicrobial resistance of the isolates was determined using Minimal Inhibitory Concentration (MIC) test (CMV3AGNF plate). To define the criteria for *Y. enterocolitica* MIC Interpretive Standards for *Enterobacteriaceae* from the Clinical and Laboratory Standard Institute were used.

Results: Antimicrobial resistance analysis showed that all *Y. enterocolitica* strains were resistant to amoxycillin/clavulanic acid, ampicillin and cefoxitin. The resistance to nalidixic acid (10.4%), tetracycline (10.4%), chloramphenicol (4.2%) and ceftriaxone (2.1%) was also observed. All tested strains were multiresistant (3 to 6 antibiotics). Multiresistant strains demonstrated resistance in most cases to amoxycillin/clavulanic acid, ampicillin and cefoxitin together (85.4%) or with other antimicrobials such as nalidixic acid, tetracycline, chloramphenicol and ceftriaxone. On the other hand, all strains tested were sensitive to ciprofloxacin, trimethoprim/sulfamethoxazole and gentamycin.

PW429 Yersinia enterocolitica - microbiological hazard in goat's milk

Anna Czubkowska[1], Jolanta Grażyna Rola[1]

[1]*National Veterinary Research Institute, Department of Hygiene of Food of Animal Origin, Puławy, Poland*

Background: *Yersinia enterocolitica* is considered as an important enteric pathogen, causing enterocolitis and other diseases in animals and humans. The consumption of goat milk has been growing and the data on the occurrence of these bacteria in Poland is still limited.

Objectives: The aim of the study was to assess the prevalence and to characterize *Y. enterocolitica* isolated from raw goat milk in Poland.

Methods: A total of 178 samples of raw goat milk were collected from 2016 to 2017 in 30 dairy farms. The presence of *Yersinia* spp. in 2016 was performed according to the reference method (ISO 10273:2003 standard). Due to the low detectability, in 2017 a modification of the standardized method was introduced: the cold culture and extend the incubation time. The species identification was performed using biochemical tests. The biotype and the serological group of isolates were determined by the reference method. To define the pathogenicity of *Y. enterocolitica* isolates multiplex PCR was performed using the *ail*, *ystA* and *ystB* gene primers encoding major virulence factors.

Results: The results obtained in 2016 indicated that the percentage of positive samples was low (14.1%). Using the modified method *Yersinia* spp. was detected in more than half of the samples (65.0%). The biochemical studies showed that all strains classified as *Y. enterocolitica* belonged to the biotype 1A. Among them 0:5 and 0:8 serotypes were distinguished, there were also non-typable isolates. Only gene encoding enterotoxin YstB was found in tested strains, *ystA* and *ail* markers were not detected.

PW430 Mycoflora and mould counts on the leaves of terminallia catappa a local food packaging material

Amina Badmos[1], eniola oni[1]

[1]*federal university of agriculture, microbiology, abeokuta, Nigeria*

Background: Leaves of *Terminallia catappa* has been used for the packaging of local foods for long, because of the assumed flavour it adds to the food it's been packed with, without considering that it might harbor harmful microorganisms that produce toxins that might be of public health concerns

Objectives: The study is aimed at determining the presence of aflatoxin producing fungi present in *Terminaallia catappa* leaves that are capable of reducing food quality and causing ill- health.

Methods: *Terminallia catappa* leaves (Almond fruit leaves) were gotten from different locations for the evaluation of fungi, mould and quantification of aflatoxins. Standard microbiological methods on coconut agar was used for the isolation of fungi, High Performance Liquid Chromatograpy (HPLC) was used for the quantification of the concentrations of aflatoxins in the leaves.

Results: The total fugal counts isolated the leaves ranged from 6 to 129 x 10^5(cfu/g). The fungal isolates were identified as *Aspergillus flavus, Aspergillus niger, Aspergillus parasiticus* and *Rhizopus sp*. Total concentration of Aflatoxin B1(AFB1) found in the leaves ranged from 3.266 to 12.507µg/kg, while Aflatoxin G1 (AFG1) ranged from 2.287 to 4.509 µg/kg in the leave sampled. The study therefore suggests cautious use of the leaves of *Terminallia cattappa* in local food packaging mainly due to the presence of toxins if public health must be protected.

PW431 Low tech method for safety analysis of fresh vegetable

Most Tahera Naznin[1], Beatrix Alsanius[2]

[1]*Swedish University of Agricultural Sciences, Department of Biosystems and Technology, Alnarp, Sweden*
[2]*Swedish University of Agricultural Sciences, Department of Biosystems and Technology, Sweden*

Background: Regular consumption of vegetable is an important part of a healthy diet and reduced risks of disease. Despite of their nutritional value sometimes food borne disease outbreaks due to consumption of fresh or minimally processed vegetables. Food-borne diseases have been evolved as a serious problem in both developed and developing countries. In developing countries of Africa and South-East Asia, foodborne diseases appeared as a very common phenomenon. Food safety field work in remote places is often challenged by events that are not foreseen in these standards; for example, power shortage or cut, instrument malfunction, and confiscated equipment. In developing countries cost of assessing food safety is also a big issue.

Objectives: The aim of this study was to find out cheap low-tech method which can be use in assessing food safety in fresh produce.

Methods: Lettuce (*Lactuca sativa*) was used as a model crop. In this experiment, we examined different extraction techniques (stomacher with filter bag as control, breadstick with filter bag and breadstick with nylon stocking) for viable count of bacteria.

Results: Results showed that the load of aerobic mesophilic bacteria was significantly different among the treatments. This research will allow for improved selection of low tech methods for assessing food safety of leafy vegetables in remote areas or for field work.

PW432 Serotype differentiation and antibiotic resistance of Listeria monocytogenes isolated from artisanal cheeses

Anna Zadernowska[1], Wioleta Chajęcka-Wierzchowska[1], Marek Zadernowski[2], Arkadiusz Zakrzewski[3]

[1]Faculty of Food Science, University of Warmia and Mazury, Department of Industrial and Food Microbiology, Olsztyn,
[2]The East European State Higher School in Przemysl, Institute of Technical Sciences, Olsztyn, Poland
[3]Faculty of Food Science, University of Warmia and Mazury, Department of Industrial and Food Microbiology, Olsztyn, Poland

Background: The microflora of cheese made from unpasteurized milk is highly similar to that of raw milk moreover during the production process, artisanal cheeses can be contaminated with pathogenic microorganisms that are transmitted by the workers and from the environment. *L*. *monocytogenes* is a frequent contaminant in the dairy farm environments.. *L. monocytogenes* is able to grow at different temperature ranging from 2 to 52 °C, and in food having low pH value (4,4) or high salt concentration (14%). At least 13 different serotypes of *L. monocytogenes* are known.

Objectives: The aim of study was to investigate the occurrence of *L*. *monocytogenes* in artisanal cheeses samples from unpasteurized cow milk and to determine their serotypes and antibiotic resistance

Methods: A total of 96 samples were obtained from several local markets in Olsztyn, Poland. Culture method of *L*. *monocytogenes* determination was based on the ISO 11290- 1:1996.
Finally, the colonies identified as *L*. *monocytogenes* were confirmed by Loop-mediated isothermal amplification (LAMP). Multiplex PCR for identification of the main serogroups (1/2a-3a,1/2b-3b,1/2c-3c,4b-4d-4e) was performed as described previously Doumith et al.(2004)

Results: Nine strains of *Listeria monocytogenes* were isolated. It was shown that six of them were resistance to ampicilin. Of the total number of strains, the most belonged to the group 1/2a-3a. This research was financed by the National Science Centre, Poland, number 2018/29/B/NZ9/00645

PW433 Fungal Metabolites Profiling in Cashew nut from Two African Countries

Modupeade Adetunji[1], Shamsideen Aroyeun[2], Mulunda Mwanza[1]

[1]*North West University, Mafikeng Campus, Animal Health Department, Mafikeng, South Africa*
[2]*Cocoa Research Institute, Ibadan, Microbiology, Ibadan, Nigeria*

Background: Infestation of food crops by mycotoxins has led to serious economical lost for food exporters at the international market due to presence of mycotoxins in the food at levels above the set regulatory limit.

Objectives: This work aims at comparing the fungal metabolites profile in cashew nut from two African countries using two different Chromatographic methods (HPLC and LCMS/MS).

Methods: Seventy-five samples of roasted cashew nut were purchased from 14 different locations in Nigeria and 10 major Supermarkets in Mafikeng, North West Province ,South Africa and were subjected to HPLC analysis for aflatoxin and Zearalenone detection and LCMS/MS analysis for multiple metabolite profiling

Results: Total Aflatoxins concentration (0.01-0.77µg/kg) and maximum Zearalenone concentration (123.23 and 788.64 µg/kg) were detected in South Africa and Nigeria nuts respectively. In contrast, LCMS/MS analysis showed that none of the major fungi metabolites but citrinin was present in detectable amount in Nigeria cashew nut (31.33µg/kg). Other microbial metabolites present in the nut were; aspergillus metabolites, Flavoglaucin, 7-Hydroxykaurenolide, Chloramphenicol. Beavericin and Bikaverin were higher in Nigeria (100% and 16.66%) as compared with the South African nut (8.33% and 8.33%), while 7-Hydroxykaurenolide and Chloramphenicol were found in all the cashew nuts. A total of 15 unspecific metabolites were recorded in the nuts (16.66-100%) for both countries. Due to the high export value of cashew nut and its high rate of local consumption as snacks, information on the metabolite profile of the nut will provide useful information for policy makers for appropriation in the country's policy

PW434 Effect of the use of chlorine usage in milking equipment cleaning procedures on raw milk microbiota

Paola Cremonesi[1], Stefano Morandi[2], Camilla Ceccarani[3], Nicola Cologna[4], Andrea Goss[4], Marco Severgnini[3], Massimiliano Mazzucchi[5], Erika Partel[5], Bianca Castiglioni[1], Alberto Tamburini[6], Zanini Lucio[7], Milena Brasca[2]

[1]*Institute of Agricultural Biology and Biotechnology, National Research Council, Lodi, Italy*
[2]*Institute of Sciences of Food Production, National Research Council, Milan, Italy*
[3]*Institute of Biomedical Technologies, National Research Council, Segrate (MI), Italy*
[4]*Consorzio dei Caseifici Sociali Trentini, Trento, Italy*
[5]*Edmund Mach Foundation, Technology Transfer Centre, San Michele all'Adige (TN), Italy*
[6]*Department of Agricultural and Environmental Sciences, University of Milan, Milan, Italy*
[7]*Breeders Association of Lombardy, Crema (CR), Italy*

Background: Sodium hypochlorite (NaClO) is generally used for disinfection of milking equipment ensuring milk hygienic quality. Different studies showed that the chlorine can affect milk microbial population, encouraging the comprehension of the relationship between the use of chlorine products and milk microbiota, which represents a key point for raw milk cheese production.

Objectives: The aim of the study was to evaluate the influence of NaClO on raw milk microbiota and the deriving whey-starter bacterial composition in Trentingrana PDO cheese production.

Methods: Three farms were involved in the study. Milk was collected thrice weekly during the NaClO usage (six-weeks period, C) and in a subsequent, analogous, nonchlorine detergent period (NC). Four-weeks interval was established between the two experimental periods. The deriving whey-starter was sampled too. Samples were submitted to microbiological analyses (Standard Plate Count, coliforms, staphylococci, Lactic Acid Bacteria) and to metagenomic analysis. To this aim, 16S rRNA gene (V3-V4 region) was sequenced by Illumina MiSeq platform. Data analyses were performed with QIIME pipeline.

Results: No significant differences were observed but higher SPC and LAB counts were recorded in milk going from period C to period NC.

The metagenomic analysis of milk showed a distinctive microbiota composition for the three farms during the whole experimental period, moreover we evidenced a significant difference in microbial population related to chlorine use.

The use of chlorine was found to affect the whey-starter population; *Lactobacillus helveticus* was predominant during period C and significantly reduced during period NC, whereas *Lb. delbrueckii* had the exact opposite trend.

PW435 The first isolation and characterisation of bacteriophages with lytic activities against environmental Escherichia coli O157:h7: potential bio-control agents

Muyiwa Ajoke Akindolire[1], Collins Njie Ateba[1]

[1]*NWU,Mafikeng Campus, Microbiology, Mahikeng, South Africa*

Background: Bacteriophages can provide alternative measures for the control of *Escherichia coli* O157:H7 that is currently an emerging foodborne pathogen of severe public health concern.

Objectives: In this study, we characterise and explore the potential of phages infecting *E. coli* O157:H7 as biocontrol agents for these pathogens.

Methods: Fifteen phages were screened against 69 environmental *E. coli* O157:H7 isolates to determine their lytic spectra. The morphologies of the phages was determined using Transmission Electron microscopy. Burst sizes and latent periods of selected phages were determined by a one-step growth experiment. The genetic diversities of the phages was determined using PFGE and RFLP analysis. The stability of phages under different chemical (salinity, and chloroform) and physical (temperature and pH) conditions were determined.

Results: Three phages (V3, V7 and V8) displayed broad lytic spectra against environmental shiga toxin-producing *E. coli* O157:H7 strains. TEM analysis of the phages revealed very high degree of similarities and were assigned to the family *Podoviridae* since they possessed isometric heads (73 – 77 nm) and short tails (20 - 25 nm) in diameter. The genome sizes were ~ 69 nm and latent periods of the 3 phages were 20 min, 15 min, and 20 min for V3, V7 and V8 respectively. The burst sizes were 374, 349 and 419 PFU/ infected cell respectively. The phages were relatively stable over a wide range of salinity, temperatures and pH values. They also displayed lytic capabilities against environmental *E. coli* O157:H7 strains and their phage cocktails may assist in bio-control applications.

PW436 First Report of a cfr/optrA-Carrying Enterococcus faecalis Isolate in Brazil

Maria Karielle Silva[1], Wellington Lima[1], Rafael Souza[1], Angela de Alencar e Silva[3], Thairine Gomides Castro[3], Felipe Zorzi[2], Pedro Henrique Filsner[2], Andrea Moreno[2], Maria Cristina Delgado[1], Ticiano Nascimento[1], Lara de Almeida[1]

[1]*Federal University of Alagoas, Brazil*
[2]*University of São Paulo, Brazil*
[3]*Federal University of Lavras (UFLA), Brazil*

Background: Oxazolidinone linezolid is one of the most important last-line antibiotics to treat infections caused by multidrug resistant Gram-positive bacteria. The spread of linezolid resistance has been driven mainly by the plasmid-borne or chromosomal 23S rRNA methyltransferase gene *cfr* and the ribosomal protection gene *optrA*. We determined the complete genome sequence of a linezolid-resistant *Enterococcus faecalis* isolate of animal origin that carried plasmids containing *cfr* and *optrA* (CP018004.1).

Objectives: We aimed to characterize the genetic environments of *cfr* and *optrA* in ST29 *E. faecalis* L9 isolated from swine, and to investigate whether or not these genes are transmissible.

Methods: Genomic DNA of *E. faecalis* L9 was sequenced (Illumina Miseq), assembled (CLC 8.0.3) and annotated (NCBI's PGAP). Conjugation by filter mating assay was performed using *E. faecalis* OG1RF as recipient strain.

Results: Sequencing of *E. faecalis* L9 revealed a *cfr*-carrying segment of 7.759 bp in which the *cfr* gene was found to be flanked upstream by the Tn*554*-related Δ*tnpB* gene, and an insertion sequence IS*1216*, while in the downstream part, *cfr* was flanked by *rec* and *pre/mob* genes. An *araC-hp-optrA* segment of 3.453 bp flanked by IS*1216* elements was also found in L9. Transconjugant OG1RF-L9 displayed two genes coding for Rep, using PlasmidFinder 1.3. The *repUS1* gene was likely involved in mobilizing the *araC-hp-optrA* plasmid, since *repUS1*8 was inserted in the *cfr*-carrying contig. Our data contribute to understanding the spread of these resistances in bacteria from food animals.

PW437 Identification of the New lnu(G)-Carrying Transposon Tn6371 in Enterococcus faecalis Isolated from Swine in Brazil

Rafael Souza[1], Maria Karielle Silva[1], Wellington Lima[1], Luis R. Sobrinho[1], Luiz R. Santos[1], Angela de Alencar e Silva[3], Thairine Gomides Castro[3], Felipe Zorzi[2], Pedro Henrique Filsner[2], Andrea Moreno[2], Maria Cristina Delgado[1], Ticiano Nascimento[1], Lara de Almeida[1]

[1]*Federal University of Alagoas, Brazil*
[2]*University of São Paulo, Brazil*
[3]*Federal University of Lavras (UFLA), Brazil*

Background: The lincosamides lincomycin, clindamycin and pirlimycin are highly important veterinary antimicrobial agents. Resistance to lincosamides in bacteria from food-producing animals, including zoonotic pathogens, has emerged due to multidrug resistance genes coding for 23S rRNA methylases, ATP-binding cassette transporters, and lincosamide nucleotidyltransferase *lnu* genes coding enzymes that inactivate the antibiotic by adenylylation. Here we report an ST710 *Enterococcus faecalis* isolate from swine that carried multiple lincosamide resistances, including a new chromosome-borne *lnu*(G)-carrying transposon

Objectives: We investigated the lincosamide resistance determinants, and described the genetic environment of Tn*6371* in the porcine *E. faecalis* strain L8, which came from a bacterial collection that exhibited high lincomycin MIC levels

Methods: Genomic DNA of *E. faecalis* L8 was sequenced (Illumina Miseq), assembled (CLC 8.0.3) and annotated (NCBI's PGAP)

Results: The 4738-bp Tn*6371* DNA segment was found to be inserted at the chromosomal *radC* gene in *E. faecalis* L8. Tn*6371* is a Tn*554* family transposon carrying an 804 bp-*linG* gene. The presence of Tn*6371*-containing circles predicted in in silico analysis was confirmed by PCR, indicating that Tn*6371* was active in *E. faecalis* L8. The upstream region of Tn*6371* was composed of a 453-bp beta-phosphoglucomutase gene, while downstream of Tn*6371* a 201-bp cold shock protein *cspA* gene was detected. In addition to Tn*6371*, *E. faecalis* L8 also carried a chromosome-borne multi-resistance cluster *lsa(E)/spw/aadE/lnu(B)*, and the lincosamide nucleotidyltransferase gene *lin*(C), which is unusual in enterococci, in a plasmid of about 90 Kb

PW438 Effects of Red Propolis in the Reduction of Biogenic Amines in Homemade Cheese

Victor Lima[1], Josicleide Silvino[1], Genildo Ferreira Júnior[1], João Victor Oliveira[1], Giselda Lira[1], Angela de Alencar e Silva[3], Thairine Gomides Castro[3], Maria Cristina Silva[1], Lara de Almeida[1], João Araújo Júnior [1], Luciano Grillo[1], Irinaldo Basílio Júnior[1], Ticiano Nascimento[1]

[1]*Federal University of Alagoas, Maceió, Brazil*
[3]*Federal University of Lavras (UFLA), Brazil*

Background: Red propolis originates from the leguminous plant *Dalbergia ecastophyllum*, which grows in the mangrove swamps of the northeastern states of Brazil. The antibacterial, antioxidant, anti-inflammatory and anticancer properties of phenolic compounds of red propolis, including cytotoxic activity on human cancer cell lines, have been demonstrated. In this study, we report the effects of red propolis on the reduction of biogenic amines and of bacteria associated with food-borne disease outbreaks.

Objectives: We investigated the activity of red propolis in reducing the biogenic amines cadaverine, phenylethylamine, putrescine and tyramine, and the coagulase-positive *Staphylococcus* spp., Enterobacteriaceae, *Salmonella* spp., and *Listeria* spp. counts (CFU/g) in homemade cheese samples.

Methods: A high-performance liquid chromatography (HPLC) assay was performed in order to detect tyramine, feniletilamine, cadaverine and putrescine in homemade cheese samples supplemented with red propolis extract (0.1%, 0.3% and 0.5%). Microbiological safety of the samples was determined according to the guidelines of the National Health Surveillance Agency.

Results: All cheese supplemented with red propolis extract showed a low concentration of biogenic amines with areas smaller than the area of the lowest detectable concentration (0.058 µg/mL). Low counts of total coliforms and *Escherichia coli* were also observed. Biogenic amines are toxic organic bases usually produced by bacteria through amino acid decarboxylation. The flavonols liquiritigenin, pinobanksin, isoliquiritigenin, formononetin, and biochanin A that could be detected in both raw propolis extract and supplemented cheese samples inhibited bacterial growth, indicating the potential role of red propolis for microbiological quality and safety of dairy products as a natural biopreservative.

PW439 Appplication of phage for Salmonella control in the food industry

Veronica Garcia[1], Matias Aguilera[1], Francisca Muñoz[1], Sofia Martínez[1]

[1]*Universidad de Santiago de Chile, Ciencia Y tecnología de los Alimentos, Santiago, Chile*

Background: One of the most important pathogens involved in the foodborne diseases is Salmonella, which are transmitted to human by poultry and the chicken meat. To avoid this, the industry has implemented several actions including disinfectants, antibiotics and vaccines for the animals. However, these actions do not be successful, and we have yet outbreaks of Salmonella. On the other side, the regulation of use of antibiotic in the primary production, valid from the 2005, show the urgent the need of new alternative for the pathogens control. In this sense, the phages have been an alternative, since are recognized as “GRAS”.

Objectives: In this job we characterized 14 phage of Salmonella for their use in food industry.

Methods: 14 phage were isolated of different sources and were characterizated microbiologically and molecularly.

Results: We show the isolation of 14 strains of phages of Salmonella from water, feces and other environmental samples, which were characterized in the host range, viral progiene and electronic microscopy. Among them the phages of Salmonella F6 and F7 showed a shorter adsorption time (30 min y 60 min, respectively) and a high viral progenie (4 fold y 200 fold respectively), the others phages showed a low viral progenie in Salmonella typhimurium. After, we selected 5 phages for test their antimicrobial activity in food. We used a mix of 5 phages in chicken meat and some phages in fish meal and we obtain a diminution of the Salmonella in both matrices using high amount of phage.

PW440 Developmentand Validation of Predictive Model for Salmonella Growth in Unpasteurized Liquid Eggs

Young-Jo Kim[1], Hye-Jin Moon[2], Soo-Kyoung Lee[3], Bo-Ra Song[1], Jong-Soo Lim[3], Eung-Jeong Heo[1], Hyun-Jung Park[1], Sung-Hwan Wee[3], Jinsan Moon[3]

[1]Ministry of Food and Drug safety, Cheongju, Korea, Dem. People's Rep. of
[2]Kyung Hee University, Department of Food and Nutrition, Seoul, Korea, Dem. People's Rep. of
[3]Animal and Plant Quarantine Agency, Gimcheon, Korea, Dem. People's Rep. of

Liquid egg products can be contaminated with *Salmonella* spp. during processing. A predictive model for the growth of *Salmonella* spp. in unpasteurized liquid eggs was developed and validated. Liquid whole egg, liquid yolk, and liquid egg white samples were prepared and inoculated with *Salmonella* mixture (approximately 3 log CFU/ml) containing five serovars (*S.* Bareilly, *S.* Richmond, *S.* Typhimurium monophasic, *S*. Enteritidis and *S*. Gallinarum). *Salmonella* growth data at isothermal temperatures (5, 10, 15, 20, 25, 30, 35, and 40 °C) was collected by the 960 hours. The population of *Salmonella* in liquid whole egg and egg yolk increased at above 10 °C, while *Salmonella* in egg white did not proliferate at all temperature. These results demonstrate that there is a difference in the growth of *Salmonella* depending on the types of liquid eggs (egg yolk, egg white, liquid whole egg) and storage temperature. To fit the growth data of *Salmonella* in liquid whole egg and egg yolk, Baranyi model was used as the primary model and the maximum growth rate and lag phase duration for each temperature were determined. A secondary model was developed with maximum growth rate as a function of temperature. The model performance measures, bias factor (B*f* 0.96-0.99) and r^2 (0.96~0.99) indicated good fit for both primary and secondary models. In conclusion, it is thought that the growth model can be used usefully to predict *Salmonella* spp. growth in various types of unpasteurized liquid eggs when those are exposed to various temperature and time conditions during the processing

* Corresponding author. Veterinary Pharmaceutical Management Division, Animal and Plant Quarantine Agency, 177 hyeoksin 8-ro, Gimcheon-si, Gyeongsangbuk-do 39660, Korea. Tel.: +82-54-912-0530; Fax: +82-54-912-0530 E-mail: moonjs727@korea.kr

PW441 Microbiological assessment of pathogenic bacteria in sprouts, dried sprouted seeds, dried powder of sprouted seeds, microgreens and baby greens in Latvia

Ieva Bergšpica[1], Elizabete Miltiņa[2], Laura Alksne[3], Irēna Meistere[1], Aija Ozola[1], Lelde Grantina-Ievina[1]

[1]Institute of Food safety, Animal Health and Environment "BIOR", Molecular Biology, Riga, Latvia
[2]University of Latvia, Microbiology and biotechnology, Riga, Latvia
[3]Institute of Food safety, Animal Health and Environment "BIOR", Microorganism Culture collection, Riga, Latvia

Background: Many sprout associated outbreaks of *Salmonella* or VTEC (verotoxin producing *Escherichia coli*) have happened in last decades therefore screening for the presence of bacterial pathogens in sprouts and similar produce is necessary. The latest VTEC outbreak caused by contaminated sprouts in EU was in Germany in 2011, affecting more than 3700 persons.

Objectives: To test for the presence of *Salmonella* spp, VTEC and *Listeria* spp. in sprouts, microgreens and similar produce in retail in Latvia.

Methods: In total, 45 samples were analysed according to following ISO (International Organization for Standardization) methods: ISO/TS 13136 (VTEC), ISO/TR 6579-1:2017 (*Salmonella* spp.) and ISO 11290-1:2017 (*Listeria* spp. and *Listeria monocytogenes*).

Results: *E. coli* was detected in 7 (15.5%) samples, *Salmonella* spp. in one (2.2 %) sample, *L. monocytogenes* was not present in any of the samples, however *L. innocua* was found in two (4.4%) samples. Verotoxin gene *stx1* was detected in two and *stx2* gene in one *E. coli* positive samples. We were able to isolate one *stx1* gene producing *E. coli* strain that was also subjected to whole genome sequencing. Isolated bacteria belonged to O11:H48 serogroup.

Identification of other bacteria present on selective media was performed with MALDI-TOF (matrix-assisted laser desorption/ionization time-of-flight mass spectrometer). In total 47 different species were determined, most of them were soil bacteria, however some have been characterized as potential human pathogens such as *Acinetobacter baumanii, Bacillus cereus, Cronobacter sakazakii, Enterobacter cloacae, E. coli, Klebsiella pneumonia* and *Pseudomonas aeruginosa.*

PW442 Microbiota and presence of foodborne pathogens in full-fat cow's, sheep's and buffalo's yellow cheese

Galina Satchanska[1;1], Tsenova Margarita[1], Stefanova Petia[2], Dicheva Violeta[2], Vatcheva-Dobrevska Rositsa[3]

[1]New Bulgarian University, Natural Sciences, Sofia, Bulgaria
[2]Hospital "Tsaritsa Yoanna", Microbiological laboratory, Sofia, Bulgaria
[3]Hospital "Tsaritsa Yoanna", Microbiological lab, Sofia, Bulgaria

Background: Cheese is rich in microbiota and more than 400 species of bacteria, yeasts and molds have been identified in it so far. Being rich in nutrients, cheese favors the growth of foodborne pathogenic bacteria.

Objectives: The aim of our study was to investigate the mi¬crobiota in three commercial full-fat cow's, sheep's and buffalo's yellow cheese as well as to search for pathogenic bacteria.

Methods: Microbiota in 1 g of cheese was assessed using selective and chromogenic media. Cheese samples were examined for presence of Clostridium, Staphylococcus, fecal enterococci, yeasts, Candida spp. and total number of bacteria. Randomly picked isolates were subcultured on Columbia agar; McConkey agar, Sabouraud and BBL CHROM agar Candida. Identification of isolates was carried out using Crystal system, VITEK, API and further tested for antibiotic resistance against six antibiotics.

Results: Our results revealed cheese's samples were negative for clostridia, *E. coli* and coliforms. Number of yeasts and Candida spp. did not exceed the limits as well. While the sheep's and buffalo's cheese were negative for staphylococci, the cow's cheese slightly exceeded the standard. Possible reason for the staphylococcal contamination, worldwide, could be the undiagnosed cow's mastitis. Isolated bacteria were further identified as *Staphylococcus simulans*, *Enterococcus avium*, *Candida krusei* and *Cryptococcus neoformans*. Abovementioned isolates demonstrated no antibiotic resistance, however, their sensitivity varied. Once ingested, these bacteria aren't risky for the general population but could be harmful to humans, causing endocarditis, bacteremia and brain abscesses in immunocompromised or chronical diseases suffering consumers, children and pregnant women.

PW443 Evaluation of Escherichia coli counts in poultry meat in Spain

Elena Gonzalez-Fandos[1], Iratxe Perez-Arnedo[1], Alba Martinez-Laorden[1]

[1]*University of La Rioja, Food Technology, Logroño, Spain*

Background: The intestinal tract of poultry could content pathogens such as *Salmonella* and *Campylobacter.* In order to reduce the poultry carcasses contamination with *Campylobacte*r spp. the hygienic status of the slaughter operations are of great importance. *E. coli* in poultry carcasses is associated to enteric contamination during processing. Thus, this bacteria could be used to indicate contamination during processing.

Objectives: The pesent study was conducted to determine the numbers of *E. coli* in poultry meat, in order to evaluate the hygienic status of two processing plants.

Methods: Two thousand and twenty two samples were taken in the period 2009-2012 in two processing plants. Samples were taken from whole carcasses, and meat portions (wings, legs, breasts). *E coli* was determined accoding to ISO 16140.

Results: *E. coli* counts below 1 log cfu/g were oberved in 11.26% and 3.92% samples from the processing plant A and B, respectively. The highest *E. coli* counts were observed in whole carcasses and wing from plant A. A decrease in *E. coli* counts were observed in both processing plants comparing the data obtained in 2009 and 2012. The *E coli* counts in the processing plant A were 3.93 log cfu/g in 2009 and 2.46 log cfu/g in 2012. The values for the processing plant B were 2.65 log cfu/g in 2012. Since some studies have associated the presence of *Campylobacter* in poultry carcasses with *E. coli* counts above 3 log cfu/g, the decrease observed in *E coli* counts is of great importance.

PW444 Effectiveness of immersion treatments with lactic and acetic acids and modified atmosphere packaging against Listeria monocytogenes in poultry

Elena Gonzalez-Fandos[1], Alba Martinez-Laorden[1], Iratxe Perez-Arnedo[1]

[1]*University of La Rioja, Food Technology, Logrono, Spain*

Background: Raw poultry is a well-recognized source of *Listeria monocytogenes* and many surveys have confirmed the presence of this pathogen on poultry. There is a great interest in reducing microbial contamination of poultry, with particular regard to reducing the levels of pathogens.

Objectives: The aim of this study was to evaluate the combined effect of lactic and acetic acid washing and packaging in modified atmospheres on the growth of *Listeria monocytogenes* on poultry legs stored at 4ºC.

Methods: Fresh chickens legs were inoculated with *Listeria monocytogenes*. After the inoculation, the chicken legs were dipped into a water solution containing 2% lactic acid (v/v) and 2% acetic acid (v/v). Control legs were treated with distilled water. Inoculated samples were packaged under different gas mixtures: vacuum, 20%CO_2/80%N_2, 40%CO_2/ 60% N_2 or air. Sensorial characteristics and *Listeria monocytogenes*, mesophiles and psychrotrophs counts were evaluated after treatment (day 0) and after storage at 4º C.

Results: Significant differences ($p<0.05$) in mesophiles and psychotrophs counts were found between the legs treated with lactic and acetic acids and the control legs after treatment. Legs washed with 2% lactic acid and 2 acetic acid and packaged in 40%CO_2/ 60% N_2 showed a significant ($p<0.05$) inhibitory effect on *Listeria monocytogenes* compared to control legs, being about 2.5 log units lower in the first ones than in control legs after 8 days of storage.

In conclusion, the combined effect of 2% lactic acid and 2% acetic acid and packaging under 40%CO_2/60%N_2 can reduce *Listeria monocytogenes* populations on fresh poultry.

PW445 Detection of enterotoxins genes in Staphylococcus aureus isolated from food handlers

Giovana Nascimento Pereira[1], Rafael Silva Rosa[1], Andre Aparecido Dias[1], Diego Júnior Santos Gonçalves[1], Camila Jesus Silva[1], Thais Batista Carvalho[1], Valeria Cataneli Pereira[1]

[1]*UNOESTE, Microbiology, Presidente Prudente, Brazil*

Background: "Pilot kitchens" are responsible for the elaboration and distribution of the meal in public schools in Brazil and the hygienic-sanitary conditions of these places are essential for the protection and promotion of students' health.

Objectives: The present study aimed at the detection of staphylococcal enterotoxin genes in *Staphylococcus aureus* isolated from the noses and the hands of food handlers of "pilot kitchens".

Methods: Classical staphylococcal enterotoxins genes (*sea*, *seb*, *sec-1* and *sed*) were investigated in 49 *S. aureus* isolated from the noses and hand of 27 food handlers from “pilot kitchens” using the PCR technique.

Results: Of the 49 samples studied, 97.9% presented the gene for at least one enterotoxin studied. The frequency of the *sea* gene was 62%, *seb* was 8%, *sec-1* was 40% and *sed* was 32%. The results are worrying, because if food contaminates during food preparation food poisoning outbreaks may occur. The need for the implementation of biosafety training for these food handlers, as well as better studies related to the impact of *S. aureus* decolonization on these professionals.

FAPESP (2018/12652-6)

PW446 revealing the influence of microbiota on the quality of fermented skate during fermentation by metagenomics and metabolomic analysis

Hye-In Koh[1], Ju-Hui Kim[1], Jong-Bang Eun[1]

[1]*Chonnam National University, Food Science and Technology, Gwangju, Korea, Rep. of South*

Background: Fermented skate also called *hongeo*, is a typical traditional fermented fish product in Korea, and it is made up of fresh skate without any additives in a ceramic jar over 1 week at room temperature.

Objectives: The aim of this study was to investigate the microbial community dynamics and metabolites changes of *hongeo* during fermentation.

Methods: The bacterial community analysis and metabolites changes of *hongeo* during fermentation was investigated using 16S rRNA gene sequencing and UHPLC-MS/MS methods.

Results: From the results, it was observed that most bacteria in fermented skate were members of the phylum *Firmicutes* and *Proteobacteria* throughout fermentation. At the genus level, *Sporosarcina* (44.57%) was predominant, followed by *Atopostipes* (22.06%) at the early fermentation phase, whereas their abundance significantly decreased to 7.67% and 8.94%, respectively, after two weeks of fermentation. At the end of fermentation, the dominant genera were *Pseudomonas* (31.66%) and *Thiopseudomonas* (20.81%). Total 341 metabolites, including amino acids, small peptides, urea cycle intermediates, nucleoside and organic acids, that contribute to both the nutritional and sensory quality of fermented skate were remarkably altered during the fermentation period. In addition, it was observed that the accumulation of urea cycle intermediate during fermentation was highly related to *Pseudomonas* and *Thiopseudomonas,* which might use this cycle to acquire their necessary nitrogen. This study provides a perspective for bridging the gap between the microbiota and metabolites in fermented skate and benefited for further optimizing production efficiency and product quality.

PW447 Roles of Penicillium roqueforti in soybean fermentation

Inhyung Lee[1], Hyun jie Kim[1]

[1]Kookmin University, Department of Bio and Fermentation Convergence Technology, Seoul, Korea, Rep. of South

Background: In traditional *meju*, a Korean fermented-soybean brick, *Penicillium* spp. are frequently observed along with other filamentous fungi. Their roles in soybean fermentation, however, are not yet understood.

Objectives: The purpose of this study is to understand the roles of *Penicillium* spp. in soybean fermentation.

Methods: The soybean fermentation characteristics of *Penicillium roqueforti* MJ1410, isolated from traditional *meju,* were compared with those of *Aspergillus oryzae* which is a well-known main fungus for *meju* fermentation.

Results: *A. oryzae* exhibited higher hydrolytic enzyme activities including protease activity than *P. roqueforti.* Accordingly, proteins degraded more quickly with *A. oryzae* than with *P. roqueforti* MJ1410. Interestingly, *P. roqueforti* MJ1410 showed much higher γ-glutamyl transpeptidase (GGT) activity than *A. oryzae* over the fermentation period. GGT produces γ-glutamyl peptides (G-peptides) that impart the kokumi flavor described as a long-lasting mouthful flavor. However, the total contents of G-peptides were higher in fermented soybeans with *A. oryzae* than with *P. roqueforti*. The lower level of G-peptides in *P. roqueforti*-fermented soybeans might have resulted from slow peptide formation due to low protease activities during the early fermentation period. One notable aspect was that the content of γ-Glu-Val-Gly that is known to most strongly promote the kokumi flavor was higher in fermented soybeans with *P. roqueforti* than with *A. oryzae*. Therefore, the possible role of *P. roqueforti* in the flavor development during soybean fermentation cannot be excluded.

PW448 Impact of bacterial surface properties on spatial distribution of S. aureus in complex emulsified matrices

Samia Almoughrabie[1;2], Valérie Poulet[2], Chrisse Ngari[2], Florence Dubois-Brissonnet[1]

[1]UMR Micalis, INRA, AgroParisTech, Université Paris-Saclay, 78350 Jouy-en-Josas, France
[2]Laboratoires Clarins, 5 rue Ampère, 95300 Pontoise, France

Background: Food or cosmetic matrices are often complex emulsified products. Evaluating and controlling the microbiological contamination of these complex products remains a major issue. The efficiency of preservation systems is generally evaluated by performing challenge-tests which monitor the evolution of an artificial contamination over time and help define the product shelf-life. The reliability of the challenge-test results can be impacted by many parameters such as the physiological state of microorganisms, the composition or texture of the matrix but also the bacterial spatial distribution.

Objectives: The aim of this study is to understand the impact of bacterial surface properties on the spatial distribution of *S. aureus* in complex emulsified products with different viscosities in order to evaluate the impact of this parameter on the reliability of challenge tests.

Methods: Calibrated suspensions of *S. aureus* were stained using fluorescent syto9 marker and inoculated in the emulsified matrices supplemented with different percentages of thickener (from 0,4 to 1,6 % acrylate copolymer). Three strains of *S. aureus* with different level of surface hydrophobicity but similar electrophoretic mobilities were used. The bacterial spatial distribution was observed by Confocal Laser Scanning Microscopy (CLSM).

Results: Results showed that the more hydrophobic the bacteria is, the more heterogeneous the spatial distribution is. Moreover, for all strains, the higher the concentration of thickener the more heterogeneous is the spatial distribution. In conclusion, the bacterial surface hydrophobicity and the thickener concentration clearly impact the bacterial spatial distribution and have to be taken into account when a challenge-test is designed.

PW449 How to Distinguish Heat Activation from Heat Damage of Bacterial Spores; Is There and Overlap?

Juan Wen[1], Jan Smelt[1], Stanley Brul[1]

[1]*University of Amsterdam, Swammerdam Institute for Life Science, Amsterdam, Netherlands*

Background: The development of the bacterial spore to a dividing vegetative cell is one of the critical factors that can determine the shelf life of food. Knowledge of the germination mechanism can contribute to an extended shelf life by either stimulating germination of spores followed by inactivation or preventing spores from germination. Selection of appropriate heating conditions can optimize the mirobiological safety and stability of the food while retaining the desirable food quality.

Objectives: To this purpose the effect of sublethal heat on the subsequent germination behaviour of *Bacillus subtilis* spores was studied.

Methods: *B. subtilis* spores were heated according to a matrix i.e. {0, 15, 30, 60, 150, 240 300 min.}*{65, 70, 75, 85 ^{0}C}. These spores were inoculated into minimal medium (MOPS) complex medium (Luria Bertani or LB)followed by optical density measurements. Apart from these turbidity studies inactivation studies were conducted by plating heat treated spores. Moreover, microscopic single cell studies were conducted and compared with the population studies

Results: OD measurements showed a continuum from 15 min. at 65 ^{0}C, where some apparent activation of germination was observed till 300 min. at 80 ^{0}C, where more than 99% was lethally injured. The microscopic observations allowed for deconvolution of the population data and showed that heterogeneity in time to start of phase darkening decreased by mild heating and increased by severe heating. In contrast, individual spore germination time (actual phase bright-phase dark transition) was mildly affected. Together the observations allowed us to develop an empirical mathematical model.

PW450 Antioxidative, antimicrobial and antibiofilm properties of Refošk wine waste extract

Katja Bezek[1], Boris Kovač[1], Mia Šivak[2]

[1]*Faculty of Health Sciences, University of Primorska, Izola, Slovenia*
[2]*Faculty of Food Technology and Biotechnology, University of Zagreb, Zagreb, Croatia*

Background: Agriculture by-products, such as grape wine waste, are mainly composted or discarded in open areas and represent a growing environmental problem. However, due to high bioactive compounds content the waste material could be reused in feed/food, pharmaceutical and cosmetic industry.

Objectives: The aim of our study was to test the antioxidative, antimicrobial and antibiofilm properties of water extracts from grape seeds and skins of typical Slovenian wine variety.

Methods: Pressed grape pomace of Refošk variety (*Vitis vinifera*) cultivated in Kras winegrowing district of Slovenia was collected. Water extracts of dried and grounded material were prepared using ultrasonic bath and were centrifuged and filtrated prior to use. The antioxidative capacity of water-soluble compounds was measured using Photochem (Analytik Jena AG). The minimal inhibitory concentrations (MIC) of water extracts for *Campylobacter jejuni* 81-176 were determined by microdilution method and PrestoBlue viability reagent. To quantify the antibiofilm activity crystal violet method was used.

Results: The antioxidative capacity of water-soluble compounds was higher in grape seeds (9.69±0.04 mmol/L) than in grape skins (2.96±0.07 mmol/L). The water extract of grape seeds was also the most effective against *C. jejuni* 81-176 with lowest MIC value (1.25 mg/mL) compared to other tested extracts. Moreover, the antibiofilm activity of both grape seeds and skins at 1/8 of MIC was shown. Our results showed the great potential of wine waste material as an inexpensive source of valuable water-soluble compounds with antioxidative capacity, as well as antimicrobial and antibiofilm activity against *C. jejuni*.

PW451 Evaluation of fixation methods for Raman Hyperspectral Imaging of Food relevant bacteria

Vicky Caponigro[1], Mariateresa Ferone[1], Amalia scannell[2], Aoife Gowen[2]

[1]*University College of Dublin (UCD), UCD School of Biosystems and Food Engineering, Dublin, Ireland*
[2]*University College of Dublin (UCD), UCD School of Agriculture and Food Science, Dublin, Ireland*

Background: The rapid detection of pathogens and spoilage microbes and other microbial contaminants in food is essential to ensure the safety of consumers. Among the emerging rapid methods for foodborne microorganism detection and identification, hyperspectral imaging (HSI) is gaining increasing attention because of its potential to take rapid, accurate and non-destructive measurements on food sample. However, monitoring bacterial growth directly on the food surface is extremely challenging due to different issues, such as signal interference by water or low microbial concentration. Making use of some common fixation methods might solve some of these challenges.

Objectives: The goal of this study is to investigate the compatibility of several widely used fixation methods for bacterial cells with subsequent analysis by Raman HSI.

Methods: Three common fixation agents, including 2.5% glutaraldehyde, 70% ethanol and ethanol/acetic acid (3:1) solution, applied to two model bacteria, *E. coli* and *B. subtilis* have been evaluated for optimal sample presentation for HSI. Raman spectra were collected using an inVia Micro-Raman confocal spectroscopy system (Renishaw, Wotton-under-Edge, Gloucestershire, UK) with a 100× objective lens, 532 nm laser (500 mW, set to 10% and 50% power two time, 10 s and 90 s). The total range was 3900–190 $cm^{-}1$. Different bacterial concentrations were also evaluated to determine the sensitivity of the method.

Results: Preliminary results showed that bacterial concentration above 10^6 cells/mL gave fluorescence-saturated signals, while below 10^5 cells/mL 10^6 the background signal was too high.

PW452 Antifungal activity of Lactobacillus paracasei in Castellano like cheese

Juliana Ramos Pereira[1], Edson Antonio Rios[2], Juliana Mareze[2], Jesús Ángel Santos[1], Teresa María López Díaz[1]

[1]*University of León, León, Spain*
[2]*University of Londrina, Brazil*

Background: *Penicillium commune* is one of the most common contaminant on cheeses responsible for spoilage and mycotoxins production. The use of lactic acid bacteria (LAB) with antifungal properties as protective cultures would be of interest, since most methods of mould control in cheeses are chemical.

Objectives: The aim of this study was to evaluate the antifungal capacity of *Lactobacillus paracasei* (Lp-25/1, isolated in previous studies) to prevent *P. commune* (M35) growth on Castellano-like cheeses

Methods: The cheeses were produced with pasteurized ewes' milk in two batches. The first batch containing the Lp-25/1 strain and a commercial starter culture and the second batch only containing the commercial starter culture (control). Both batches were surface-inoculated with the mould and ripened for 60 days. Mould growth was analyzed by photographs and enumeration of mould and yeast in Chloramphenicol Glucose Agar at the end of the ripening.

Results: The application of Lp-25/1 resulted in a delayed growth of *P. commune* until the end of the experiment compared to the control cheeses. Mean mould and yeast counts in the cheeses with Lp-25/1 were 6.53 log cfu/ml and in those without the strain were 7.22 log cfu/ml and a visual analysis showed a clear reduction in the amount of mycellium in both batches. There was a significant difference in the means of mould and yeast counts ($P < 0.05$). These results show that Lp-25/1 has the potential to be used as a bioproctector culture in cheese.

PW453 Extraintestinal Pathogenic and Antimicrobial-Resistant Escherichia coli from retail meat in Sicily

Giorgia Caruso[1], Anna Giammanco[2], Cinzia Cardamone[1], Giuseppa Oliveri[1], Chiara Mascarella[2], Giuseppina Capra[2], Sara Cannella[2], Miriam Sciortino[2], Ignazio Arrigo[2], Maria Rita Tricoli[2], Teresa Maria Assunta Fasciana[2]

[1]*Institute for Experimental Veterinary Medicine of Sicily , Palermo, Italy*
[2]*University of Palermo, Pro.Mi.Se, Palermo, Italy*

Background: Extraintestinal infections due to *Escherichia coli* are an important cause of morbidity, mortality, and increased health care costs. Meat products are knowed to be vehicles for transmitting antimicrobial-resistant and extraintestinal pathogenic *E.coli* (ExPEC) to consumers.

Objectives: To reassess this hypothesis in the current era of heightened concerns about antimicrobial use in food animals, we analyzed 46 samples of meat purchased from retail stores in Palermo in order to obtain quinolone-resistant *E. coli* isolates.

Methods: Strains were screened for their phylogenetic groups, ST131-associated single nucleotide polymorphisms (SNPs) and then typed by ERIC-PCR.
Their sets of virulence factors, namely *kpsMII, papA, sfaS, focG, iutA, papC, hlyD* and *afa* genes, were investigated and their fluoroquinolone-resistance determinants evaluated.

Results: The data obtained show a high prevalence of multidrug resistance patterns in the Palermo area, with 28% of the isolates having virulence factor genes typical of ExPEC strains. No B2 group or ST131 strains were detected. Moreover, 20% of our isolates showed positivity to all the plasmid-mediated quinolone resistance (PMQR) determinants, showing a potential to transfer these genes among other bacteria. Therefore, these data underline the possibility that animals food, and specifically poultry in particular, may be a significant source of resistant bacterial strains, posing a potential zoonotic risk.

PW454 A Model Smart Quality Assurance and Safety System for Fresh Poultry Products - QAPP

Chrysoula Tassou[1], Konstantinos Koutsoumanis[2], Yannis Vagiannis[3], Ilias Sarigiannidis[4], George-John Nychas[5]

[1]Hellenic Agricultural Organisation DEMETER, Institute of Technology of Agricultural Products, Greece
[2]Aristotle University of Thessaloniki, Dept. of Food Science abd Technology, Greece
[3]Vertoyo OE, Greece
[4]Kotino AEBE, Greece
[5]Agricultural University of Athens, Lab. of Food Microbiology and Biotechnology, Greece

Background: Nowadays there is a need for the food industry to predict the freshness /safety profile of perishable products, effectively control and reduce food losses and improve compliance with EU Regulations on microbiological criteria.

Objectives: The vision of "QAPP" project is the development of an "Intelligent" Management System for Product-specific Quality and Safety, focused on poultry products and being supported by an integrated ICT services platform that incorporates analytical methods, data and decision-support tools.

Methods: The QAPP will include a novel cloud-enabled data and model storage system, which will allow the integration of heterogeneous information derived from food microbial ecosystem throughout its production and distribution chain. These heterogeneous data will consist of (i) conventional microbiological analyses (ii) spectroscopic profiling data and surface chemistry spectra from non-invasive analytical sensor devices (iv) data mining methods and prediction of microbial population or quality. Poultry-based products will be used as model systems to validate the approach.

Results: This holistic approach will establish a next-generation monitoring reference system for food safety & quality through the integration and analysis of complex structured or non-information data (Big data) that enable real time predictions for safety and freshness profiling. This integration will provide comprehensive maps of important food traits, as well as microbial predictive models to aid in decision-making on food quality and safety.

Acknowledgment: This research has been co-financed by the European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innovation, under the call RESEARCH–CREATE-INNOVATE (project code: T1EDK-04344)

PW455 Metagenome sequence analysis of the natural fermentation starter nuruk

Ju Yeon Song[1], Ki Young Yoon[1], Jeong-Ah Seo[2], Min-Jung Kwak[1], Jihyun F. Kim[1;3]

[1]Yonsei University, Department of Systems Biology and Division of Life Sciences, Seoul, Korea, Rep. of South
[2]Soongsil University, School of Systems Biomedical Science, Seoul, Korea, Rep. of South
[3]Strategic Initiative for Microbiomes in Agriculture and Food, Seoul, Korea, Rep. of South

Background: *Nuruk* is a natural fermentation starter used to produce Korean traditional alcoholic beverages. The Korean rice wines are made from rice and various grain flours with the starter *Nuruk*. Diverse enzymes are synthesized from the *nuruk* microbiota and are involved in saccharification and fermentation, during which raw materials of cereals are digested and small compounds affecting the flavor of liquor are produced.

Objectives: Composition of microorganisms is a key factor that influences the quality of alcoholic beverages. It is presumed that the flavor of traditional alcoholic beverages depends on the kinds of raw materials for *nuruk* production. Since various flavors in alcoholic beverages might result from different microbial composition, we compared microbial communities in different *nuruk* samples through microbiome approaches.

Methods: *Nuruk* samples were collected in different fermentation steps. To exclude the undesirable wheat gDNA, a purification method for preparation of the metagenomic DNA was established and then the extracted DNA was subject to metagenomic sequence analysis. This involves microbiota analysis using 16S rRNA gene/ITS region sequences and microbiome analysis using whole metagenome shotgun sequencing data.

Results: Microbial and functional diversity were monitored through comparative metagenomic analysis of different *nuruk* samples and fermentation steps. We could observe microbial dynamics in *nuruk* by fermentation time and obvious difference of the microbial structures by raw materials. Genes on metabolic pathways were analyzed and the results indicated that various flavors of fermentation products were related to different composition of microbiota and metabolic genes.

PW456 Impact of commensal bacteria on Listeria moncytogenes biofilms produced under simulated meat processing conditions

Timothy Ells[1], Stephanie Haddad[2], Michele Elliot[1], Todd Smith[2], Louise Deschênes[3], Tony Savard[3]

[1]Agriculture and Agri-Food Canada, Kentville Research and Development Centre, Kentville, Nova Scotia, Canada
[2]Acadia University , Department of Biology, Wolfville, Nova Scotia, Canada
[3]Agriculture and Agri-Food Canada, Saint-Hyacinthe Research and Development Centre, Saint-Hyacinthe, Quebec, Canada

Background: *Listeria monocytogenes* is a major problem for the food industry. As part of its survival strategy *Listeria* produces biofilms, which protect against harsh conditions found in food processing settings. Most biofilm studies have been conducted with monocultures using a single growth medium. This does not represent real life events, where bacteria are subjected to fluctuating conditions and polymicrobic biofilm communities dominate.

Objectives: To determine the impact of two commensal bacteria on biofilm-formation by *L. monocytogenes* grown under simulated meat processing conditions (SMPC).

Methods: Biofilms were cultivated on two surfaces (stainless steel and high density polyethylene) over a 12-day regimen of SMPC. To compare biofilm dynamics, *L. moncytogenes* was grown in monoculture, dual-species cultures with *Lactobacillus plantarum* or *Pseudomonas fluorescens*, or in tri-species cultures with both commensals. Biofilms were assessed by viable cell counts and scanning electron microscopy (SEM). Finally, minimum biofilm eradication assays (MBEC) were conducted to determine the protective capacity of the biofilms against different santizers.

Results: Relative to its monoculture biofilms, *L. monocytogenes* paired with the *Lactobacillus* strain displayed lower initial attachment levels ($P < 0.5$); however, after 12 days, viable *Listeria* in these dual cultures were similar to monocultures (~6.5 $\log_{10}$ CFU/cm^2). When the pseudomonad was present, in dual or tri-species cultures, *Listeria* day-12 numbers significantly increased ($P < 0.5$) by up to 1 log. *Pseudomonas* biofilms displayed significantly higher MBECs for both benzalkonium chloride and hydrogen peroxide, an attribute likely afforded by substantial production of extracellular polymeric substances (EPS) as visualized by SEM.

PW457 Effect of Different Coagulants on the Amino acid content of Soft cheese (wara) Produced from Sheep milk

Ayodele Ogunlade[1], Victor Oyetayo[2]

[1]*The Federal Polytechnic, Ado-Ekiti, Food Technology , Ado Ekiti, Nigeria*
[2]*Federal University of Technology Akure, Microbiology, Akure, Nigeria*

Background: Soft cheese (wara) is an unripened cheese consumed in several parts of West Africa due to its various nutritional qualities. Soft cheese, a coagulated product of raw milk is usually produced from cow milk using *Calotropis procera*. Although soft cheese can also be produced from other animals using other coagulants.

Objectives: This study therefore sought to assess the effect of the different coagulants such as *Calotropis procera, Carica papaya*, lemon juice and steep water from cereals (maize, millet and sorghum) on the amino acid content of soft cheese produced from sheep milk.

Methods: Raw milk sample was collected from sheep and processed into soft cheese by these coagulants and the amino acid composition of the sample was carried out using standard methods.

Results: The result revealed that *Calotropis procera* coagulated soft cheese has the highest essential amino acid content Leucine (10.21g/100g), while steep water from millet coagulated soft cheese has the lowest essential amino acid content methionine (0.72g/100g). However, lemon juice coagulated soft cheese has the highest non essential amino acid glutamic acid (16.27g/100g) in all the cheese samples.

PW458 Pigment Production, Charaterization and Enzamatic Properties of Endophytic Fungi for Beneficial Traits

Madira Coutlyne Manganyi[1], Thierry Regnier[2], Christ-Donald Kaptchouang Tchatchouang[1], Cornelius Carlos Bezuidenhout[3], Collins Njie Ateba[1]

[1]*Department of Microbiology, North-West University—Mafikeng Campus, South Africa*

[2]*Department of Biotechnology and Food Technology, Tshwane University of Technology, South Africa*

[3]*Unit for Environmental Sciences and Management, North-West University-Potchefstroom Campus, South Africa*

Background: Pigments are used as colouring additives in various essential and profitable industries such as textile, pharmaceutical, food, cosmetics, plastics, paint, ink, photographic and paper. New sources of natural pigments are getting particular research interests due to the toxicity produced by synthetic colouring agents. Apart from the enormous antibacterial applications, fungi may provide a readily available alternative source of natural pigments.

Objectives: The fungal strains were tested to determine whether they are capable of producing pigments suitable for dyeing purposes.

Methods: The fungal strains (n=193) from indigenous medicinal plants *Sceletium tortuosum* and *Pelargonium sidoides*. The growth and pigment production of the fungi was optimised to obtain highest yield. The correlation between pigments production and enzyme activity was determined. HPTLC was used to quantify the fungal pigments.

Results: Thirty-one (n=31) were capable of producing pigments with a broad spectrum of colours. Cell concentration of the 68% was very high as compared to moderate cell concentration of 29% and low cell number of 3%. Strong lipase (65%) and amylase (61%) activities were exhibited by the endophytic fungi. Reddish colonies were observed due to the presence of laccase enzymes. This enzyme lacked in most investigative fungi resulting in a low 13% positive. These enzymes are an attribute of biochemical processes resulting in production of protein and degradation of polysaccharides. The HPTLC confirmed that pigments can be a product of more the one colour. Due to the toxic nature of artificial colourants, there is a need to finds better, safer, affordable alternatives.

PW459 Biodiversity and enzymatic activity of wood degrading fungi isolated from Transilvanian heritage wooden churches

Alina Sirghi[1], Ionela Sarbu [2,3], Irina Gheorghe[4], Luminita Marutescu[1], irina balotescu [2], ion blajan[5], Mateescu Venus [5], Fotea Petronela[5], Zaharia Daniela[6], Cristea Violeta Corina[7], ionut pecete[7], Veronica Lazar[1], Mariana Carmen Chifiriuc[1]

[1]*University of Bucharest, Faculty of Biology, Microbiology Department, Research Institute of the University of Bucharest*
[2]*Research Institute of the University of Bucharest*
[3]*University of Bucharest, Faculty of Biology, Genetics Department*
[4]*University of Bucharest, Faculty of Biology, Microbiology Department, Research Institute of the University of Bucharest, Romania*
[5]*The Romanian Peasant Museum*
[6]*University of Bucharest, Faculty of History*
[7]*Synevo Central Laboratory, Medicover Bucharest, Romania*

Background: The 17th to 19th century wooden churches from Transylvania are an iconic symbol for the Romanian national heritage. As a result of exposure to natural elements, and poor preservation techniques, many of such edifices from Hunedoara and Arad counties ended up in high state of degradation, mainly due to wood degrading fungi.

Objectives: to constitute a revelant collection and associated database of fungal species involved in the biodeterioration of heritage wood monuments and identify the fungal exoenzymes the are involved in wood decay. These strains will be used to screen for mycoparasitic origin biofungicides which can be used as an alternative to the chemical fungicides.

Methods: Samples were collected from a total of eight wooden churches, using cotton sterile swabs, inoculated on specific culture media (Potato Dextrose Agar and Rose Bengal) and identified by classical (macro / microscopic) and automated (MALDI-TOF) methods. After growth on liquid cultered media, the supernatant was filtered and enzymes were quantified through specific techniques. *Trichoderma* sp. strains were tested for their antagonistic activity.

Results: The strains were identified by MALDI-TOF as *Alternaria alternata*, *Aspergillus clavatus*, *A. niger, A. montevidensis, A. flavus, A. ochraceus, A. pseudoglaucus, Trichoderma longibrachiatum, T. orientale, Rhizopus oryzae, R. sexualis, Fusarium incarnatum, Penicillium crysogenum, P. digitatum, P. italicum, P. verrucosum, P. brevicompactum, P. corylophylum, Curvularia palescens, Byssochlamys spectabilis, Arthridium phaeospermum (Ascomycota)* and *Schyzophyllum commune* (*Basidiomycota*). Some *Trichoderma* strains exhibited a strong antagonistic activity against other tested species and were strong producers of chitinase and glucanase.

Acknowledgments: PCCDI 52 /2018 Research Project.

PW460 Dermatophytosis in the Czech Republic: results of a five-year epidemiologic study

Petr Hamal[1], Vit Hubka[2], Adela Cmokova[2], Radim Dobiáš[3], Nada Mallatova[4], Pavlina Lyskova[5], Karel Mencl[6], Helena Janouskovcova[7], Jirina Stara[8], Jana Dolezalova[9], Lucie Svobodova[1], Miroslav Kolarik[10]

[1]University Hospital Olomouc, Microbiology, Olomouc, Czech Republic
[2]Charles University, Faculty of Science, Botany, Prague, Czech Republic
[3]Institute of Public Health, Bacteriology and Mycology, Ostrava, Czech Republic
[4]Regional Hospital Ceske Budejovice, Parasitology and Mycology, Ceske Budejovice, Czech Republic
[5]Institute of Public Health, Parasitology, Mycology and Mycobacteriology, Prague, Czech Republic
[6]Regional Hospital Pardubice, Clinical Microbiology, Pardubice, Czech Republic
[7]University Hospital Plzen, Microbiology, Plzen, Czech Republic
[8]General University Hospital Prague, Dermatovenerology, Prague, Czech Republic
[9]Regional Hospital Liberec, Clinical Microbiology and Immunology, Liberec, Czech Republic
[10]Czech Academy of Sciences, Institute of Microbiology, Laboratory of Fungal Genetics and Metabolism, Prague, Czech Republic

Background: Dermatophytes are the most successful fungi causing superficial mycoses in humans and animals. Dermatophytosis, a disease caused by anthropophilic or zoophilic dermatophytes are usually mild in primary hosts. If transmitted to other hosts, however, they induce intense inflammatory reactions. There are considerable geographic variations in the prevalence of infections and range of pathogen species.

Objectives: A five-year multicenter epidemiologic study was conducted to determine the frequency of dermatophyte species as etiological agents of skin mycoses in the Czech Republic. This was the first epidemiological study of that extent using molecular genetic methods to identify dermatophyte species.

Methods: The study, carried out from 2011 to 2016, included seven microbiology centers. Identification of isolates was performed based on their micro- and macromorphological features and subsequently confirmed by genetic methods, namely PCR-fingerprinting with the M13-core primer and, in ambiguous cases, ITS rDNA sequencing.

Results: In mycoses of the glabrous skin (tinea corporis, n=2361), the rates of anthropophilic (53.53%) and zoophilic (42.53%) species were relatively similar; the rest were geophilic species (3.94%). The most frequent anthropophilic species was *Trichophyton rubrum*; the most common zoophilic dermatophyte was *Trichophyton benhamiae*, followed by *Microsporum canis*. Infections of the scalp (tinea capitis) were rather rare (n=79) and were mostly caused by zoophilic species (75.9%). The most frequent pathogens were *M. canis* (40.5%) a *T. benhamiae* (31.6%). The overwhelming majority of onychomycoses were due to anthropophilic dermatophytes, mainly *T. rubrum*. Zoophilic and geophilic dermatophytes were responsible for only 0.6% and 0.5% of infections, respectively.

PW461 Activity of Schinus lentiscifolius ethanolic extract associated to the fungus Beauveria bassiana against larvae of the tick Rhipicephalus (B.) microplus.

Rafaela Fantatto[1], Rodrigo Sorrechia[1], Amanda Figueiredo[2], Alessandro Minho[2], Ana Carolina Souza[2], Rosemeire Pietro[1]

[1]School of Pharmaceutical Sciences, São Paulo State University, UNESP, Department of Drugs and Medicines , Araraquara, Brazil
[2]Embrapa Pecuária Sudeste, São Carlos, Brazil

Background: Infestations by ectoparasites, mainly by the tick *Rhipicephalus (Boophilus) microplus*, represent a great economic obstacle to cattle breeding. The intense massive use of synthetic acaricides has produced ticks that are resistant to the molecules available in the market. Thus, studies seeking effective alternatives should be encouraged.

Objectives: To evaluate derivatives of natural products in association with entomopathogenic fungi against *R.* (*B.*) *microplus* larvaes.

Methods: *Beauveria bassiana* fungus, kept in BDA medium, was scraped and placed in saline solution adjusting the concentration to 10^7 conidia/mL. The plant extract was obtained by maceration. The sensitivity tests of *R. (B.) microplus* larvae were performed according to the technique developed by FAO, where approximately 100 larvae of the species were placed on filter paper measuring approximately 10 x 8 cm, impregnated at concentrations of 10 to 1.25% associated with fungal suspension and controls. These papers impregnated with the extracts were folded into a "sandwich", sealed and incubated at 27 ° C and 80% relative humidity. The triplicates were read after 5 days of incubation with the help of a vacuum pump, adapted to differentiate live and dead larvaes.

Results: The results showed that in the highest concentration mortality was 15.46%. Despite the low percentage of mortality presented, it should be considered that the concentrations used were small. Another point to be highlighted is that the methodology is proposed for the detection of resistance to commercial acaricides and here is adapted to natural products and fungal associations.

Support: Fapesp, CNPq

PW462 Evaluation of aflatoxin production potentials of fungi isolated from feedlots of animals kept for food production: a polyphasic approach

Stephen Abiola Akinola[1], Collins Njie Ateba[1], Mulunda Mwanza[2]

[1]North West University Mafikeng Campus, South Africa, Department of Microbiology, Mmabatho, South Africa
[2]North West University Mafikeng Campus, South Africa, Department of Animal Health, Food Safety and Security Niche, Mmabatho, South Africa

Background: Production of secondary metabolites (mycotoxins) by fungi in food/feed substrate constitutes a menace to the safety and health of consumers.

Objectives: This study investigate the presence of aflatoxin producers and aflatoxin production potentials of *Aspergillus* species in feedlots of animals kept for food production using a polyphasic approach.

Methods: Forty-five isolates belonging to *Aspergillus* species were assessed for aflatoxin production potentials using yeast extract sucrose (YES) agar and β-Cyclodextrin Neutral Red Desiccated coconut agar (conventional), amplification of aflatoxin regulatory genes; *aflR*, *aflJ*, *Nor (aflD)*, *Ver (afl*M*)*, *Omt-A*) and toxin quantification on Thin Layer Chromatography (TLC) and High Performance Liquid Chromatography (HPLC). *Aspergillus flavus* ATCC 259622^{TM} was used as positive control while *Saccharomyces cerevisiae* and nuclease free water were used as negative and non-template control respectively.

Results: Percent occurrence of fungal isolates ranged from 2 – 42%; with *Aspergillus flavus* (42%) been highest. A total of 86.04% had *afl*D, *aflM* (58.14%), *omt-A* (72.09%), *aflR* (88%) and *aflJ* (100%). About 23 (51%) of *Aspergillus* species produces aflatoxin on YES and β-CDNRDCA and were confirmed on thin layer chromatography. HPLC results showed total aflatoxin (AFTOT) concentration of selected toxigenic fungi ranged from 6.77 – 71453 µg/g, AFB1 (3.76 - 70288 µg/g), AFB2 (6.77 – 242.50 µg/g), AFG1 (1.87 -745.30 µg/g) and AFG2 (1.67 -768.52 µg/g). Feedlots of animals reared for food are reservoirs of aflatoxigenic fungi which could pose threat to public health. This study also shows that more than one approach is necessary for the identification of toxigenic strains.

PW463 Screening of potent Bacillus species for sustainable biocontrolling of aflatoxigenic Aspergillus flavus

Pramod Poudel[1], Priyanka Thakur[1], Sushmita Dura[1], Om Prakash Panta[1], Santosh Khanal[1], Tika Bahadur Karki[1]

[1]*National Collge, Microbiology, Nepal*

Background: Aflatoxins are the fungal toxins produced predominantly by *Aspergillus flavus*, occurring in cereal and grain products. The emergence of these toxins are one of the crucial factors responsible for life-threatening disease such as liver cancer.

Objectives: To isolate and screen potent *Bacillus* species from soil and compost samples to sustainably inhibit the growth of aflatoxigenic *A. flavus.*

Methods: Twenty-five samples (soil and compost) were randomly collected from different places of Nepal. The selective growth of *Bacillus* was done by pre-heating the samples at 80°C for 10 minutes. The isolation of *Bacillus* was done on Nutrient Agar and identification of the organism was carried out by performing Gram's staining, spore staining and biochemical tests. Screening of isolates for antifungal activity was done by dual-culture technique on Potato Dextrose Agar and mycelial inhibition was observed.

Results: Fifty four isolates were Gram positive, spore former, rods, catalase positive and tentatively identified as genus *Bacillus.* Among them, only two selected isolates ($\mathrm{BH_{11}S}$ and $\mathrm{B_5S}$) effectively inhibited the growth of *Aspergillus flavus* MZ1. The zone of growth inhibition by $\mathrm{BH_{11}S}$ and $\mathrm{B_5S}$ were 3mm and 13mm respectively. These selected isolates were able to utilize glucose, xylose, fructose, mannose, lactose and maltose. The above results indicate that these bacterial isolates can be used as antifungal agent for inhibiting the growth of aflatoxigenic. In future, *Bacillus* species isolated from soil could be used for the biocontroliing the fungal pathogens with possible reduction in postharvest loss.

PW464 Biofilm formation and adhesion capability of Candida spp. clinical isolates

Zhanna Sobkova[1], Nataliia Popenko[2]

[1]*National Military Medical Clinical Center, Microbiological , Kiyv, Ukraine*
[2]*Ukrainian Military Medical Academy, Military Therapy, Kiyv, Ukraine*

Background: Yeasts of genus Candida are among ten most common pathogens in hospitals surgical profile accounting for 17% of the total number of infectious complications in the intensive therapy units (ITU).

Objectives: To evaluate the pathogenic potential of Candida isolates from patients' urine, sputum, blood and throat in the cases of nosocomial ITU infections by assessing yeasts ability for adhesion and biofilm formation (BF).

Methods: Adhesive properties of isolated strains of microorganisms were studied on human erythrocytes of Rh-positive blood group 0(I) by Brilisa. BF capability was carried out according to Romanova.

Results: The strains able for adhesion and therefore with higher pathogenic potential dominated. There were 30% of highly-adhesive strains and 33% strains with medium adhesion. None-adhesive strains were detected only for C. albicans and C. glabrata, and low-adhesive only for C. albicans. C. kruzei, C. tropicalis, C. sake, C. lusitaniae, C. parapsiolis demonstrated high- and medium-adhesiveness with the maximum values for C. krusei and C. sake species. The ability of C. non-albicans isolates for high and medium adhesion was 3 times higher than for C. albicans suggesting C. non-albicans yeasts are becoming more aggressive as nosocomial ITU infections agents. Highly- and medium-adhesive strains were detected in urine and blood, whereas low- and no adhesive strains were isolated from throat and sputum smears. The highest BF ability (0.55 ± 0.12 U OD) was shown by the isolates from urine, and the lowest ability by yeast isolated from throat (0.32 ± 0.03 U OD).

PW465 Differential response of the Candida albicans trehalose-deficient mutants to Amphotericin B and Micafungin

Juan Carlos Argüelles[1], Vanessa Pujante[2], Alejandra Argüelles[1], M. Luz Muñoz[1], Ruth Sánchez-Fresneda[1], Genoveva Yagüe[3], José Pedro Guirao-Abad[2]

[1]Universidad de Murcia, Facultad de Biologia, Murcia, Spain
[2]Universidad de Murcia, Murcia, Spain
[3]Hospital Clínico Universitario Virgen de la Arrixaca, Facultad de Medicina-Universidad de Murcia, Murcia, Spain

Background: The non-reducing disaccharide trehalose has been proposed as a promising target for the development of new antifungal compounds.

Objectives: To gain insight on this hypothesis, we have studied in the highly prevalent human pathogen *Candida albicans* the degree of susceptibility shown by null mutants disrupted in the two genes coding for the sequential steps of trehalose biosynthesis, namely trehalose synthase (*TPS1*) and trehalose phosphatase (*TPS2*) to the widely prescribed antifungals, Amphotericin B (AmB) and Micafungin (MF).

Results: Whereas *tps1Δ* cells were highly sensitive to AmB exposure, they displayed a significant level of resistance to MF. Notably, the opposite phenotype was recorded in the *tps2Δ* mutant. In turn, MF induced a significant level of endogenous ROS production in the parental SC5314 strain and *tps2Δ* cells, whereas ROS formation in the *tps1Δ* mutant was virtually undetectable. The level of endogenous ROS positively correlated with the mitochondrial activity. Only AmB was able to promote a net intracellular synthesis of trehalose in the parental strain SC5314, being absent in *tps1Δ* cells and showing a slight level in *tps2Δ*. Furthermore, the capacity to form biofilms was drastically reduced in both *tps1Δ* and *tps2Δ* mutants after the treatment with AmB, although it was increased in *tps1Δ* cells after the addition of MF.

Conclusions*:* Our data allow to strength the idea of using trehalose as a promising target for antifungal therapy.

PW466 Biological Control of Post-Harvest rot pathogens of Banana and Plantain fruits with Trichoderma species

Ayodeji Adebesin[1]

[1]*Federal Polytechnic Ede Nigeria, Science Laboratory Technology , Ede, Nigeria*

Background: Banana and Plantain fruits are prone to spoilage by moulds that are usually controlled with chemical preservatives which could be hazardous to consumers.

Objectives: The ability of *Trichoderma* in controlling post-harvest rot pathogens of Banana and Plantain fruits was determined.

Methods: *Trichoderma harzianum*, *Trichoderma viride* and *Trichoderma* sp. 1 isolated from different soil Banana farm soils in Ede, Nigeria were used for *in-vitro* antagonism rot pathogens on dual cultures by pairing. Pathogenicity of the rot pathogens from the fruits were determined and the effect of the coating of fruits with spores of the antagonists as preservative was done.

Results: Fungal species isolated from banana and plantain fruits were: *Colletotrichum*, *Fusarium*, *Geotrichum*, *Curvularia*, *Cladosporium*, *Mucor*, *Verticillum* and *Aspergillus* species. These organisms produced characteristic rot lesions when inoculated on uninfected banana and plantain fruits. The best *in-vitro* inhibitory effects on the pathogens was produced when *Trichoderma* species were introduced into the plates two days before the pathogens and 100% reduction was obtained with *Colletotrichum*, *Fusarium*, *Cladosporium* and *Verticillum* after 4 day of the pairing. *Mucor* species was slowly inhibited and 71.5% radial growth reduction was obtained on the 10th day after pairing. *Trichoderma harzianum* displayed the best control ability on the plantain coated with spore suspension of the antagonists as the fruit retained its freshness and texture throughout the test period. *Trichoderma* species could be suitable as biopesticides in preservation of fruits and for controlling post-harvest rots of banana and plantain fruits.

PW467 Molecular identification and characterization of Botrytis cinerea associated to native plants in Coquimbo Region Chile

Luis Castillo[1], Anna Notte[1], Verónica Plaza[1], Lila Olivares[1], Barbara Marambio[1], Evelyn Silva-Moreno[2]

[1] Universidad de La Serena, Laboratorio de Bioquímica y Biología Molecular, Departamento de Biología, La Serena, Chile
[2]Universidad Autónoma de Chile, Instituto de Investigación Biomédica, Santiago, Chile

Background: *Botrytis cinerea* is a phytopathogenic fungus can cause significant crop losses in the worldwide agricultural industries. However, its presence in the endemic flora in the Coquimbo Region has not been studied yet.

Objectives: In order to determine whether the native plant in the Coquimbo Region is present *Botrytis*, fifty native plants were analysis.

Methods: A total of 31 putative *Botrytis* were isolated and phenotypic and genetic characterized. The ITS of rDNA was amplified using ITS1/ITS4 primers and then sequenced. ITS analysis of this isolate revealed that it corresponded to genus *Botrytis*. For further confirmation, nuclear protein-coding genes (G3PDH, HSP60, and RPB2) were sequenced and showed 100% identity against *B. cinerea.* On the order hand, we investigated the presence and frequency distribution of the transposable elements *Boty* and *Flipper*, virulence capacity and fungicide-resistant in the isolates obtained.

Results: The results indicate that 84% of the isolates presented both transposable elements, *Boty* and *Flipper*, indicating that the most prevalent genotype was *transpose*. In addition 31.3% of the isolates showed substantially reduced virulence in apple fruit in compare to B05.10 strain. According to fungicide resistance studies, the results indicate that one isolate presented low resistant level (LR) to a commercial fungicide fenhexamide and five isolates presented different grade of sensibility to boscalid. The results provided here contribute to our fundamental understanding of the presence of *B. cinerea* in the native plant in the Coquimbo Region and the potential negative effect can be produce against to agriculture and the native plant.

PW468 construction and characterization of the t-type and mt-type solopathogen strains

Yu Jinmeng[1], Wenqiang Xia[1], Zihong Ye[1], yafen zhang[1]

[1]*china jiliang university, college of life science, hangzhou, China*

Background: *Zizania latifolia* were formed by the infection of *Ustilago esculenta* to stimulate the expansion of its stem base. *U. esculenta* is a typical dimorphic fungus, which has two forms: yeast and mycelial. The transformation of two types of *U. esculenta* is closely related to the formation of *Z. latifolia*, the genetic mating of *a* and *b* locus has been shown to regulate conversion of the dimorphism of fungi in a variety of Phytophthora fungi. Two haploid cells could fuse to form a binuclear mycelium when they differ at *a* locus, while binuclear mycelial cells are pathogenic only when they are different at locus *b*.

Objectives: When *Z. latifolia* was planted in the fields, it is easy to form grey Jiaobai. There are many brown teliospores in the grey Jiaobai, and teliospores are formed by the diploid formation of *a* and *b* genes. We constructed two solopathogen strains to reduce the formation of teliospores and make *Z. latifolia* pregnant.

Methods: We isolated compatible haploid strains from grey Jiaobai and white Jiaobai named Ue T14, Ue T55, Ue MT10 and Ue MT46 respectively. Using the UeT14 strain DNA as a template, the *mfa1.2* and *bE1* sequences were cloned, and the mature fragment was ligated into the linearized pUMa932 plasmid to obtain the pSP107 plasmid. Then linearized the plasmid and respectively transferred it into UeT55 and UeMT46 protoplasts, to obtain solopathogen strains .

Results: We obtained two solopathogen strains: UeTSP and UeMTSP, which can infect *Z. latifolia* and make it pregnant.

PW469 Heavy metal tolerance and removal potentials of fungi isolated from waste battery dump-site

Daberechi Mela[1], Chukwudi Anyanwu[1], Chukwudi Onwosi[1]

[1]*University of Nigeria, Nsukka, Microbiology, Nsukka, Nigeria*

Background: Waste battery originating from electronic appliances has resulted in the introduction of large amount of toxic heavy metals into the environment. The resulting metal contamination threatens the microbes, plants and humans.

Objectives: Realizing the biosorbing ability of microbes associated with polluted environments, it is important to isolate and characterize the fungal isolates associated with waste battery. The tolerance pattern of the isolates towards the heavy could be evaluated.

Methods: Soil sample from waste battery dumpsite was screened for the heavy metal-tolerant fungi. Identification of metal-tolerant fungi was done by morphological characterization and was confirmed by gene sequencing of the internal transcribed spacer regions. The specific growth rates (μ) metal-tolerant isolates were determined using Gompertz, logistic and exponential models. The inhibitory effects of the heavy metals on the growth of the isolates were modeled by Luong and Tyagi models.

Results: The isolates were identified as: *Trichosporon* sp., *Candida phangngaensis*, *Sporobolomyces* sp., *Cunninghamella bertholletiae* and *Penicillium citrinum*. The tolerance pattern of *C. bertholletiae* and *P. citrinum* were Cu^{2+}> Pd^{2+} > Cd^{2+} and Pd^{2+} > Cu^{2+} > Cd^{2+}, respectively. Parameter estimates from modeling the growth of *Cunninghamella* sp. under varying concentrations of Cd^{2+} gave μ, lag phase, and cell diameter as 0.06, 5.06 and 0.59cm, respectively, using modified Gompertz model (R^2=0.989). Growth of *P. citrinum* under inhibitory concentrations of Cu^{2+} resulted in μ_{max} and specific inhibitory concentration were 2.98 and 2094mg/L, respectively, using Luong model (R^2=0.986). This investigation suggested that fungi could be effectively utilized in removal of heavy metals from wastewater.

PW470 Characterization of Trichoderma dfg10 deletion mutant altered in dolichol saturation

Elżbieta Gryz[1], Urszula Perlińska-Lenart[1], Sebastian Piłsyk[1], Agata Lipko[2], Ewa Świeżewska[2], Joanna Kruszewska[1]

[1]*Institute of Biochemistry and Biophysics Polish Academy of Sciences, Laboratory of Fungal Glycobiology, Warszawa, Poland*
[2]*Institute of Biochemistry and Biophysics Polish Academy of Sciences, Department of Lipid Biochemistry, Warszawa, Poland*

Background: Dolichols, polyisoprenoid alcohols with a saturated α-isoprene unit are found in all living organisms. These compounds are components of cell membranes and structures belonging to the secretory system. Previous studies of dolichols from *Aspergillus niger* revealed that in addition its dolichols were polysaturated. Our studies confirmed that polisaturation of dolichols could be characteristic for filamentous fungi. In *S. cerevisiae* saturation of dolichols is catalyzed by dolichol saturase encoded by dfg10 gene. In this study we have examined function of dfg10 protein in *Trichoderma*. This enzyme could have additional activity catalyzing saturation of the double bonds located at the omega end of dolichol.

Objectives: The aim of this study was to show that the dfg10 gene encoded dolichol saturase.

Methods: Isolation of dolichols from *T. reesei* and Δ DFG mutant. HPLC analysis of dolichols. Analysis of activity of cis-prenyltransferase and dolichol-dependent enzymes (synthase DPM (dolichyl phosphate mannose), endogenous DPM synthase). Analysis of protein glycosylation.

Results: HPLC analysis revealed that Δ DFG mutant synthesized polysaturated polyprenols not found in the wild-type strain. The amount of polyprenols produced by the Δ DFG mutant was higher compared to the *T. reesei* strain. Activity of cis-prenyltransferase was 3.5-fold lower in the mutant compared to the parental strain, while activity of dolichol-dependent enzymes was not changed. The total amount of carbohydrates bound to the proteins secreted by the Δ DFG mutant after 120 h of cultivation was 24% lower compared to carbohydrates from proteins secreted by the parental strain.

PW471 Identifying specialisation and pathogenic trade-offs in metacommunities of fungi exposed to a persistent organic pollutant

Celso Martins[1], Adélia Varela[1], Céline Leclercq[2], Oscar Nuñez[3], Tomáš Větrovský[4], Jenny Renaut[2], Petr Baldrian[4], Cristina Silva Pereira[1]

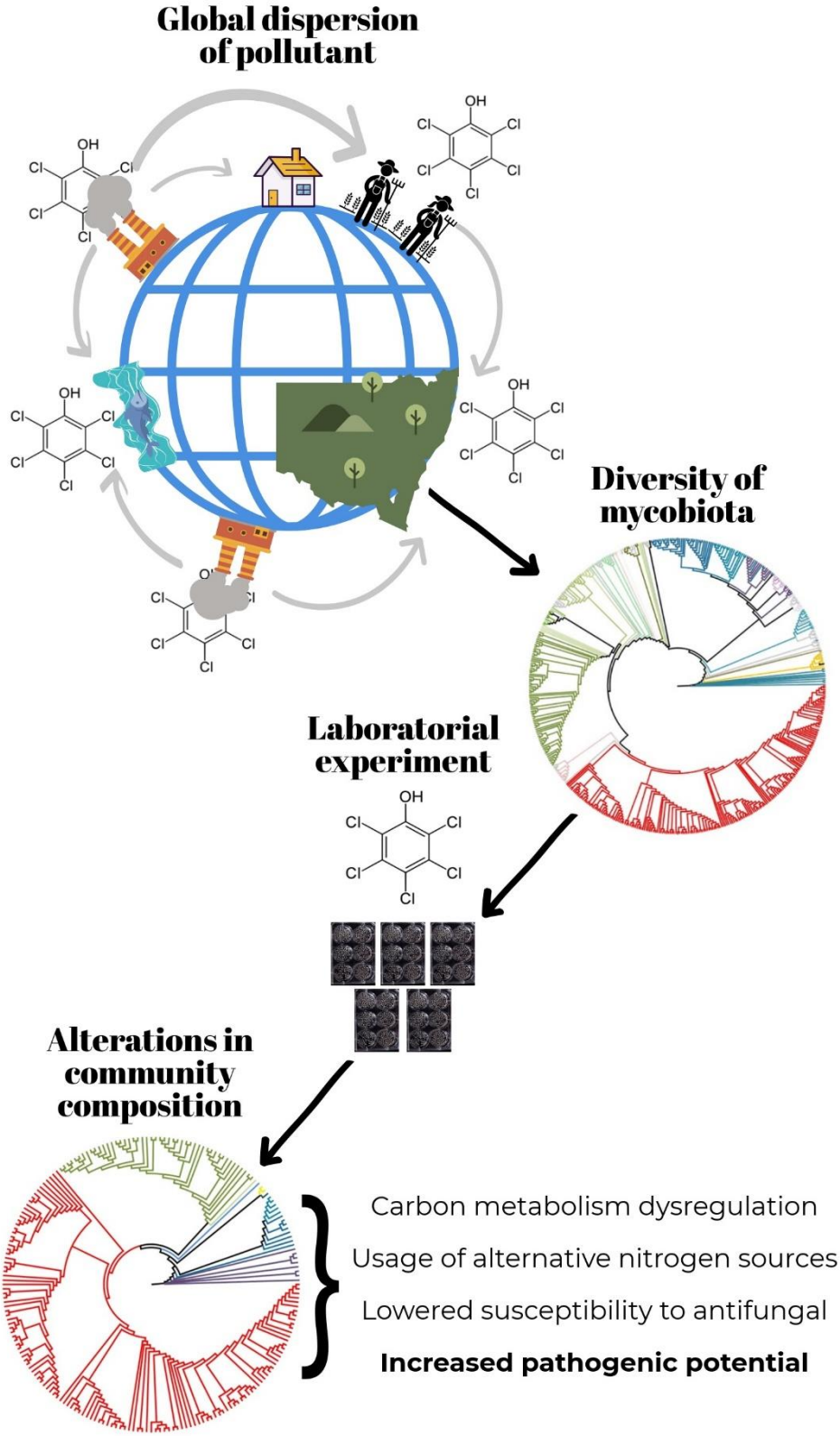
[1]*ITQB Nova, Biology Division, Oeiras, Portugal*
[2]*Luxembourg Institute of Science and Technology (LIST), Esch-sur-Alzette, Luxembourg*
[3]*Universitat de Barcelona, Barcelona, Spain*
[4]*Institute of Microbiology of the ASCR, v. v. i., Czech Republic*

Background: The impacts of man-made chemicals, in particular of persistent organic pollutants, are multifactorial as they may affect the integrity of ecosystems, alter biodiversity and have undesirable effects on many organisms. We have previously demonstrated that the belowground mycobiota of forest soils acts as a buffer against the pollutant pentachlorophenol (PCP). However, the trade-offs made by mycobiota to mitigate PCP remain cryptic.

Objectives: Demonstrate that exposure to PCP leads to alterations in the composition and functioning of the fungal community, which are associated with increased pathogenicity.

Methods: We have used multi-well cultivations to allow the establishment of metacommunities of fungi in the presence or absence of PCP. Liquid cromatography and mass spectrometry (MS) were used to analyse the pollutant decay and ensuing products, respectively. The functional traits of the metacommunity were investigated using Biolog FF microplates and MS-based proteomics. The composition of the metacommunities was studied using amplicon sequencing.

Results: Exposure to pentachlorophenol led to alterations in the composition and functioning of the metacommunity. Proteomic and physiological analyses showed that the carbon and nitrogen metabolisms were particularly affected. This dysregulation is possibly linked with higher pathogenic potential of the metacommunity following exposure to PCP, supported by the secretion of proteins related to pathogenicity. Our findings provide additional evidence for the silent risks of environmental pollution, particularly as it may favour the development of threatening pathogenic trade-offs in fungi. Future studies will focus on testing *in-vivo* the virulence of airborne fungal spores upon exposure to environmental pollutants.


Global dispersion of pollutant
Diversity of mycobiota
Laboratorial experiment
Alterations in community composition
Carbon metabolism dysregulation
Usage of alternative nitrogen sources
Lowered susceptibility to antifungal
Increased pathogenic potential

PW472 Film-forming Agents as Potential Barriers to Fungal Skin Infections

Kaho Ho[1], Begona Delgado-Charro[1], Albert Bolhuis[1]

[1]*University of Bath, Dept of Pharmacy and Pharmacology,, Bath, United Kingdom*

Background: Superficial fungal infections are one of the most common causes of human diseases caused by dermatophytes. Although the infections are mild and rarely life-threatening, they are frequently recurring, and the incidence has increased continuously especially in the urban region. Simultaneously, there is a growing resistance to antifungal drugs, exposing in particular the immunosuppressed patient to higher levels of risk. The aim of the project is to develop a physical barrier that can prevent the early stages of infection to the skin, to avoid development of antifungal resistance and cross-contamination.

Objectives: To identify the effectiveness of film-forming agents to prevent and treat fungal skin infections.

Methods: An *ex vivo* model using porcine skin was developed to study the potential of film-forming agents in preventing and treating fungal infections caused by dermatophytes (the mould *Trichophyton rubrum* and *T. interdigitale*, and the yeast *Candida albicans*). To study the effects of film-forming agents on these species, cell viability assays, confocal and electron microscopy were used. The mechanism of action of the film-forming agents were determined using QTOF-LCMS, NMR and ICP-OES.

Results: Two cationic polymers used in pharmaceutical and cosmetic products inhibited the growth of *T. rubrum*, *T. interdigitale* and *C. albicans* on porcine skin. Viability assays and microscopy imaging indicated that the polymers have a fungicidal activity against the *Trichophyton* species, while they act as fungistatics against *C. albicans*. These cationic polymers appeared to coat the cells and inhibit fungal growth by removing the carbohydrate content and iron chelation.

PW473 Fusarium on French garlic, etiology and characterization of an emerging disease

Paul Chrétien[1], Magali Duffaud[1], Cindy Morris[1], Christel Leyronas[1]

[1]*INRA, Pathologie Végétale, Montfavet, France*

Background: Yearly, France produces more than 20000 tons of garlic representing 3500 producers and 3000 ha. Since the mid-2000s and in spite of high demanding production specifications, a phytopathogenic fungus belonging to genus *Fusarium*, causes damage during storage leading to root and bulb rots. The annual losses have a major impact on the viability of the French industry.

Objectives: Two major objectives were to :

- Elucidate the etiology of the disease on garlic
- Develop a test to evaluate the aggressiveness of strains

Methods: Strains were isolated from the two major French production areas (South West and South East) during the 2017 and 2018 production campaigns. Eleven garlic varieties on more than 90 plots were used. All tested cloves were surface-sterilized with NaOCl and fungi were allowed to develop on PDA.
To evaluate aggressiveness of each strain, twelve surface-sterilized cloves soaked in $1.0*10^6$ spores/mL were used. All cloves were placed separately at 23°C at saturated humidity for 18 days. Symptoms were noted with rating scale from 0 (absence of symptoms) to 5 (clove entirely brown). Every 2 days, Area Under the Disease Progress Curve was calculated for each clove.

Results: In total, 1580 strains were successfully isolated, 262 identified (98% *F. proliferatum* and 2% *F. oxysporum*) and 48 tested for their aggressiveness. All varieties seemed to be infected equally, some plots more than the others. All tested strains shows aggressiveness on garlic, from 60 to 140% as compared to a reference strain.

PW474 Expression of soluble and attached virulence factors in non candida albicans strains isolated from different clinical specimen

Dunya Al Kurjiya[1], haifa najie[1]

[1]*Faculty of Biology, Department of Botany-Microbiology, București, Romania*

Background: : non-*Candida albicans* (NCA) have recently emerged as an important cause of human infections and an essential feature for host colonization and establishment of fungal diseases is the capacity of NCA to adhere tightly to different surfaces, including host tissues

Objectives: The study was investigation of virulence factors expression in NCA clinical isolates

Methods: a total of 68 NCA Species identification was performed by the automated Vitek-2 system. Evaluation of adhesion to cellular substratum was carried out using the Cravioto adapted method and the Hep-2 cells. The adhesion patterns & index were evaluated by microscopic examination. Fungal attachment to the inert substrata and biofilm production were assessed by using the micro titer method. The fungal isolates were also tested for the production of haemolysins and extracellular hydrolytic enzymes, i.e.: phospholipase (pz) and proteinase (prz).genetic support of adhesins (agglutinin-like sequence proteins(als) and secretory aspartyl proteinase(sap) was investigated by PCR

Results: the fungal isolates exhibited an enhanced ability to colonize the cellular substratum, with a prevalent localized-diffuse adherence pattern. All fungal isolates adhered to the inert substratum. Biofilm formation was higher in C. tropicalis, C. famata and C. krusei isolated from tracheal secretions, sputum and other secretions. Pz and prz production was detected in all isolates. The genetic analysis revealed that a high number of isolates harbored the genes belonging to als and sap adhesins families. The most prevalent were als8 and als5, sap2, sap3 and sap10, these genes being detected in more than half of the NAC isolates.

PW475 Effect of temperature in the growth and study of hydrophobicity in Candida auris clinical isolates from different geographical regions

Elías Cortés-Acosta[1], Bibi Sara Azahar-Climent[1], Elian Mora-Jimenez[1], Piet W.J. de Groot[2], Eulogio Valentin-Gomez[1]

[1]*University of Valencia, Microbiology, Burjassot (Valencia), Spain*
[2]*Regional Center for Biomedical Research, Molecular Mycology, Albacete, Spain*

Background: The emerging multidrug-resistant pathogenic yeast *Candida auris* causes life-threatening invasive infections and shows a capacity for hospital transmission that is uncommon in other *Candida* species. Because very little is known about the physiology of *C. auris* we have studied the effect of incubation temperature on growth, phosphomannan content, and hydrophobicity of clinical *C. auris* isolates from different countries in comparison to *C. albicans* SC5314.

Objectives: The aim of this work was to analyze if there is variability in surface hydrophobicity and phosphomannan content among different clinical isolates of *C. auris.* To determine the optimal growth temperature for these experiments, we first analyze growth at different temperatures.

Methods: Growth temperature was studied using a drop test (DOI: 10.1093/femsyr/foy124) and allowing growth for 24 hours at 28ºC, 37ºC and 42ºC. Hydrophobicity was determined as described (DOI: 10.1016/j.fgb.2012.01.010): cell suspensions with an OD_{600nm}= 1.0 were mixed with xylene and after phase separation the OD_{600nm} of the aqueous phase was measured. Phosphomannan was determined as the Alcian blue binding capacity as described (DOI: 10.1128/EC.1.3.420–431.2002).

Results: The best growth temperature for *C. auris* was 37ºC, though growth speed variability was observed between isolates. Most (95%) *C. auris* isolates contained less phosphomannan than *C. albicans*. Half of the *C. auris* showed higher surface hydrophobicity while the other half was less hydrophobic than *C. albicans*. All tested South American isolates were at least four and Indian isolates two times more hydrophobic than *C. albicans*. One Indian isolates was seven times more hydrophobic.

PW476 Molecular diagnosis of als genes as virulence factor in non-albicans candida species isolated from immune compromised patients

Dunya Al Kurjiya[1]

[1]*Faculty of Biology, Department of Botany-Microbiology, Bucharest, Romania*

Background: Non-*Candida albicans* (NCA) have recently emerged as an important cause of human infections and an essential feature for host colonization and establishment of fungal diseases is the capacity of NCA to adhere tightly to different surfaces, including host tissues and agglutinin-like sequence (ALS).

Objectives: The purpose of this study was to assess by genotypic methods the adherence features of 71 (NCA) clinical strains isolated from a hospital in Bucharest

Methods: used clinical strains of 71 non- *Candida albicans spp*. The strains were identified by Vitek and confirmed by Api *Candida* and they isolated from various clinical specimens and cultured in Sabouraud's dextrose broth . Specimens collection From january to July 2018, were isolated from samples of immunocompromised patients (wound secretion,sputum,blood, urine, rectal, treacle secretion and vaginal secretion). The genetic support of the adherence genes was investigated by PCR. Simplex and multiplex PCR were performed on genomic DNA in order to establish the ALS 1,2,3,4,5,6,7,8,9,10.

Results: In analyzed strains The most frequently encountered adherence genes of non albicans *Candida spp* were for *C.glabrata* (28%) and *C. tropicalis* (22%) but the lowest number for *C.parapsilosis* (4%) with high significant different (P<0.07) between non albicans *Candida spp*. Which showed the high percentage of *C. glabrata* was (27%), *C. tropicalis* (23%) and low percentage was for *C. parapsilosis* (5%). The highest percentage ALS gene was for ALS 7 (80.28%) followed by ALS 10 and ALS 8 (35%), ALS 1 (18%) and lowest ALS 3 (10%) with high significant different (*P<0.05*) between ALS genes..

PW477 Fungi from karst caves in China

Zhi-feng Zhang[1;2], Lei Cai[1]

[1]*State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China*
[2]*College of Life Science, University of Chinese Academy of Sciences, Beijing, China*

Background: Karst caves are characterized by darkness, lower temperature, higher humidity, and oligotrophy. Studies have revealed that caves encompass a high fungal diversity, while almost all of the investigations are based on culture method and rarely documented in China.

Objectives: 1) to explore the fungal diversity in karst caves in China; 2) to determine the factors regulating fungal communities in caves; 3) to hypothesize the origin of cave fungi.

Methods: Fifteen karst caves in Southwest China were investigated based on culture and culture-independent method.

Results: In total, 3763 fungal strains belonging to five phyla, 294 genera and 881 species were isolated, of which, 53 new species had been described based on morphology and phylogeny. For metabarcoding analyses, approximately 10,000 fungal OTU were obtained after data processing. Comparing with air and carbonate samples, soil and water harboured higher diversity and distinct communities. Substrate and cave were revealed to be the key determinant of fungal community in caves. Fungal community of air and water between the cave and outside cave environment are similar, while, that for rock and soil samples are significantly different. Molecular dating showed that divergence time of seleved potential true cave fungi (no late than 7.2 Mya) was much earlier than the forming time of these two investigated caves (3.5–4 Mya). The geographic history of caves appeared to be too short for fungal speciation and fungi in caves were more likely to be travelers from other environments.

PW478 Effect of fungicide propiconazole on the yeast-like symbiotes in brown planthopper (BPH, Nilaparvata lugens Stal) and its role in controlling BPH infestation

Xuping Shentu[1], Xiaolong wang[1], Yin Xiao[1], Yu Xiaoping[1]

[1]Zhejiang Provincial Key Laboratory of Biometrology and Inspection & Quarantine, College of Life Sciences, China Jiliang University, Hangzhou, China

Background: Yeast-like symbiotes (YLS), harbored in the abdomen fat-body of the brown planthopper (BPH), *Nilaparvata lugens* Stål, are vital to the growth and reproduction of their host.

Objectives: In order to prove the feasiblity of BPH infestation manipulation on rice by inhibiting YLS using fungicide.

Methods: Propiconazole was injected into the hemolymph of BPH thorax to investigate its effect on YLS, especially the dominant species, *Hypomyces chrysospermus*, and their host.

Results: Propiconazole markedly reduced the number of YLS and *H. chrysospermus* in hemolymph and fat body, thereby leading to an obvious higher mortality and lower fecundity of BPH than the negative control (PBS, phosphate buffer solution). After microinjecting propiconazole, the survival rate of BPH nymphs at the 5th instar was significantly lower than that obtained after PBS treatment.8 days after propiconazole microinjection, the BPH survival rate dropped to 40%, only half of BPH survival rate treated with PBS. For female adults (1 day old), there were significant differences in the survival rates between BPHs treated with propiconazole and those treated with PBS at days 5–8. The fecundity of BPH decreased significantly by microinjecting propiconazole and averaged only 229 eggs per female, which was 20% less than that of the negative control. Furthermore, we reared BPH on the susceptible variety TN1 sprayed with propiconazole. The number of YLS and *H. chrysospermus* in BPH obviously declined. Subsequently, the survival rate and fecundity of BPH significantly decreased. Therefore, inhibiting YLS using fungicides was a novel and effective way to control BPH infestation.

PW479 Transformation of a Pseudogymnoascus strain of Antarctic origin

Inmaculada Vaca[1], Anai Diaz[1], Pablo Villanueva[1], Vicente Oliva[1], Felipe Vega[1], Carlos Gil-Duran[2], Francisco Fierro[3], Renato Chavez[2]

[1]*Universidad de Chile, Faculty of Sciences, Chile*
[2]*Universidad de Santiago de Chile, Faculty of Chemistry and Biology, Chile*
[3]*Universidad Autonoma Metropolitana, Departamento de Biotecnologia, Mexico*

Background: *Pseudogymnoascus* is a fungal genus prevalent in most of Antarctic environments, but to date it has been poorly studied. One of the main reasons of this lack of study is the absence of a transformation system allowing genetic manipulation.

Objectives: The purpose of this work is the development of transformation systems for the Antarctic fungus *Pseudogymnoascus* sp.

Methods: Two transformation methodologies were developed: protoplast-mediated transformation (PMT) and electroporation of germinated conidia. In both cases, plasmid pAN7-1, containing a hygromycin B resistance cassette under the control of regulatory sequences of *Aspergillus nidulans*, was used. For PMT, protoplasts were produced using lytic enzymes from *Trichoderma harziarum* and β-glucuronidase, whereas transformation was performed using PEG 3350 and recovery conditions of transformed protoplasts of 24 h at 15 °C in CM-sorbitol medium. On the other hand, the electroporation of germinated conidia was performed using lithium acetate buffer and pulses of 1,000 Volts.

Results: Using PMT protocol, transformation frequencies between 3.8-6.3 transformants/µg of DNA were achieved. On the other hand, the electroporation of germinated conidia yielded transformation frequencies between 3-10.6 transformants/µg of DNA. The results here shown represent the first report of transformation of Antarctic strains of *Pseudogymnoascus*, and will allow an important advance for the genetic manipulation of these filamentous fungi. This work was supported by projects INACH RG_15-14 and FONDECYT 1150894.

PW480 Effect of culture medium and 5-azacytidine on the expression of secondary metabolism genes in the fungus Pseudogymnoascus verrucosus

Renato Chavez[1], Vicente Oliva[2], Anai Diaz[2], Pablo Villanueva[2], Carlos Gil-Duran[1], Inmaculada Vaca[2]

[1]*Universidad de Santiago de Chile, Faculty of Chemistry and Biology*
[2]*Universidad de Chile, Faculty of Sciences*

Background: Fungi from the genus *Pseudogymnoascus* produce secondary metabolites with novel chemical structures. Despite this, the secondary metabolism of this fungal genus has been poorly studied.

Objectives: We studied the effect of the culture medium and the epigenetic modifier 5-azacitidine (5-AZA) on the transcriptional pattern of five genes (*gymB*, *gymC*, *gymD*, *gym722* and *gym36*) from secondary metabolism of *Pseudogymnoascus verrucosus,* isolated from an Antarctic marine sponge.

Methods: The strain was cultivated in five culture media (PDB, YES, YES-NaCl, CYA-sucrose, CYA-lactose), the RNA was extracted at three different times (5, 8 and 10 days) and the expression of the genes was assayed by RT-PCR. To determine the effect of 5-AZA, the fungus was grown in PDB plus 5-AZA (50 or 500 mM). 5-AZA was added at two different times of the culture (0 or 7 days).

Results: Expression of *gymB*, *gymC* and *gymD* was detected in all culture media assayed, but expression of *gym722* was never detected. Regarding *gym36*, expression was observed only in PDB at 8 and 10 days of culture. Addition of 500 mM of 5-AZA repressed the expression of *gymB*, *gymC* and *gymD*. In contrast, the addition of 50 mM of 5-AZA increased 1-2 fold the expression of *gymC*. Interestingly, 5-AZA added at the beginning of the culture induced the production of a reddish pigment. These results indicate that the culture medium and 5-AZA have effects on the transcription pattern of the secondary metabolism genes in *P. verrucosus*. Work supported by INACH RG_15-14, FONDECYT 1150894, and DICYT-USACH.

PW481 Role of the HOG1 pathway in the Candida albicans response against Amphotericin B and Micafungin

Juan Carlos Argüelles[1], José Pedro Guirao-Abad[1,2], Ruth Sánchez-Fresneda[1], Elvira Román[2], Vanessa Pujante[1], Jesús Pla[1], Rebeca Alonso-Monge[2]

[1]*Área de Microbiología, Facultad de Biología, Universidad de Murcia, Spain*
[2]*Departamento de Microbiología y Parasitología, Unidad de Microbiología, Facultad de Farmacia, Universidad Complutense de Madrid, Spain*

Background: The MAP-kinase HOG1 pathway plays an essential role in the pathobiology of the opportunistic yeast *Candida albicans*.

Objectives: We have planned to analyze the hypothetical involvement of Hog1 in the *C. albicans* protective defense against two antifungals currently prescribed in clinical therapy, namely Amphotericin B and Micafungin (MF).

Results: The MICs calculated for the parental strain (RM100) and the homozygous *hog1Δ* null mutant was identical for MF in both strains (0.016 mg/L), but it was half for AmB in *hog1Δ* (0.03 mg/L) compared to RM100 cells (0.06 mg/L). At elevated doses, *hog1Δ* was more susceptible to AmB (0.5 mg/L) than RM100, whereas the two cell types showed similar sensitivity to MF (0.05 mg/L). AmB induced a large endogenous level of ROS formation measured by flow cytometry together with the simultaneous increase of the mitochondrial membrane potential, while MF failed to stimulate both mechanisms. Remarkably, the genes coding for the antioxidant activities catalase and SOD were highly expressed upon exposure to AmB. In turn, AmB but not MF triggered a significant rise of the oxidant scavenger trehalose. Moreover, AmB activated the phosphorylation of Hog1 and Mkc1 while MF caused Mkc1 phosphorylation.

Conclusions: Our results support that generation of an intracellular oxidative stress is a contributory factor to the fungicidal action of AmB. They also point to the differential involvement of the Hog1 MAPK in the *C. albicans* response against AmB and MF.

PW483 Dye decoloration in airlift bioreactor using liquid and solid culture enzymes of white rot fungus Trametes versicolor CBR43

Hee Wook Ryu[1], Wooyoung Yang[1], Jinyoung Lee[1]

[1]*Soongsil University, Departement of Chemical Engineering, Seoul, Korea, Rep. of South*

Background: Many white-rot fungi have the ability to decolorize and decompose a variety of dyes and are interested in their use. Liquid culture enzymes are widely used, but it is necessary to utilize solid phase culture enzyme which does not require separation process.

Objectives: This study is to investigate the possibility of a new fungal decolorization process by directly injecting the sawdust medium-based solid culture of white rot fungi into the dye waste wastewater and also possible to find applicable operating conditions.

Methods: *Trametes versicolor* CBR 43, a white-rot fungus capable of decoloration of various dyes, was isolated. The dye decolorizing ability of sawdust-based solid cultures and liquid culture enzymes of CBR 43 strain was evaluated by airlift bioreactor. The effects of pH, temperature, and solid culture concentration on dye decolorization were investigated. The decolorizing ability of the solid culture enzyme was evaluated by repeated injection of dye.

Results: The decolorization rate of the acid blue dye was 400-650 mg $L^{-1}h^{-1}$ in the liquid enzyme. The rate increased linearly with increasing concentration of the solid culture medium and was 930 mg $L^{-1}h^{-1}$ at 3 wt%. Compared with the liquid culture enzyme, the solid culture enzyme had faster dye decolorization rate and longer decolorization ability. The rate was constant in the temperature range of 5-40^{o}C, and the optimum pH was 4-7. These results indicate that the white-rot mycelium cultured on sawdust medium-based medium can be applied to the dye decolorization process.

PW484 Isolation and Antibacterial analysis of Filamentous Fungi from Roudsea Wood and Mosses National Nature Reserve

Teresa Dias[1], Marta Filipa Simões[2]

[1]Faculty of Sciences, University of Lisbon, Department of Biology, Portugal
[2]Edge Hill University, GEMM-Group for Extreme and Marine Microbiology, Department of Biology, United Kingdom

Background: Fungi have evolved the capacity to synthesize metabolites in order to adapt to the variety of niches they inhabit. They are a wide and underexplored source of potential bio-products. Roudsea Wood and Mosses National Nature Reserve (NNR) is an area of conservation at Cumbria which encompasses several different environments, including extreme ones like marshes and bogs, being ecologically diverse and ideal locations for fungal prospection.

Objectives: This work intends to investigate the diversity of filamentous fungi in the underexplored Roudsea Wood and Mosses NNR based on isolation assays and screen these fungal strains for their potential antibacterial activity.

Methods: Several locations (8) with different edaphic parameters were chosen for collection of water (4) and soil/sediments (7) samples. These were characterised regarding their physical and chemical parameters. The samples were then serial-diluted and inoculated in Malt extract agar (MEA), and incubated at 27ºC. Fungal colonies were selected, and re-inoculated until axenic cultures were obtained, followed by macro and micro-characterisation and screening of selected strains for antibacterial activity.

Results: A total of 59 filamentous fungi were isolated. Being the highest load of isolates found in samples associated with rhizosphere and with the highest acidity (pH=3.08-4.04). Of those, 12 phenotypically different strains were chosen for further studies, macro and micro characterisation, and antibacterial essays. It was possible to identified three isolates at genus level based on morphology, and five isolates demonstrated promising results against Gram-positive (*Micrococcus luteus*, and *Staphylococcus aureus*) and -negative bacteria (*Escherichia coli*, and *Pseudomonas aeruginosa*).

PW485 The diversity and characteristics of Penicillium from intertidal zone in South Korea

Myung Soo Park[1], Seung-Yoon Oh[1], Young Woon Lim[1]

[1]*School of Biological Sciences, Seoul National University, Seoul, Korea, Rep. of South*

Background: The genus *Penicillium* is commonly isolated from various terrestrial and marine environments and play an important ecological role as a decomposer. Although reports of *Penicillium* in the marine environment are increasing, there is no comprehensive study of how many species are present and what their role is.

Objectives: Our aims is to understand *Penicillium* diversity and their ecological roles in intertidal zone

Methods: We investigated the diversity and community structure of *Penicillium* from intertidal zone using used both a culture-dependent and -independent approach and tested for halotolerance, enzyme activity, and polycyclic aromatic hydrocarbons (PAHs) degradation.

Results: 103 *Penicillium* species from intertidal zone were identified using isolation, ITS-NGS, and BenA-NGS methods. Although BenA-NGS is efficient for detecting Penicillium in intertidal zone, some *Penicillium* species uniquely detected in isolation and ITS-NGS methods. The *Penicillium* community displayed a significant degree of variation relative to season (summer and winter) and seaside (western and southern coast). Many *Penicillium* isolated in this study exhibited cellulase and protease activity, and/or degradation of PAHs. Penicillium as decomposer is thought to play an important role in nutrient recycling and pollutant degradation in the intertidal zone.

PW486 Chemical characterization of Lavandula dentata essential oil cultivated in Chile and its antibiofilm effect against Candida spp.

Andrea Müller-Sepúlveda[1;2], José A. Jara[2], Carolina Belmar[2], Pablo Sandoval[2], Rocío Santander Meyer[3], Raúl Quijada[4], Sidnei Moura e Silva[5], Mario Díaz-Dosque[2], Alfredo Molina-Berríos[2]

[1]Universidad de O´Higgins, Institute of Agronomic and Veterinary Sciences, Rancagua, Chile
[2]Universidad de Chile, Laboratory of Pharmacology, Institute for Research in Dental Sciences, Faculty of Dentistry. , Santiago, Chile
[3]Universidad de Santiago de Chile, Department of Enviromental Sciences, Faculty of Chemistry and Biology, Santiago, Chile
[4]Universidad de Chile, Faculty of Physics and Mathematics Science, Santiago, Chile
[5]Universidade de Caixas do Sul, Laboratory of Biotechnology of Natural and Synthetic Products, Biotechnology Institute, Brazil

Background: *Lavandula dentata* is an endemic plant in Chile with a well-known antimicrobial activity and low toxicity. However, its effect against fungal biofilms has been poorly described. *Candida albicans* and *Candida tropicalis* are the most common human fungal pathogen and with the increase in resistance rates worldwide the search for pharmacological alternatives is warranted. In addition, *Candidas spp* related infections often involve biofilms development, which are highly resistant respect to their planktonic counterparts. In this context, essential oils could be promising as antifungal treatment.

Objectives: The aim of this work was to evaluate the antifungical and antibiofilm effect of *Lavandula dentata* essential oil against *Candida albicans* and *tropicalis.*

Methods: In this study, we chemical characterized an essential oil from *Lavandula dentata* by Gas Chromatography-Mass Spectrometry. Antifungal effect was evaluated on *C. albicans* (ATCC 90029-10231) and *C. tropicalis* (ATCC 750) by disk diffusion assay and minimal inhibitory concentration was obtained through microdilution assay. Effect of essential oil on adhesion ability of *Candida spp* was determined through crystal violet assay. Finally, antibiofilm effect was evaluated through scratch assay.

Results: Our results identified as the main constituent of the essential oil to monoterpenoid eucalyptol (60%). The extract presented an antifungal effect with a minimal inhibitory concentration (MIC) lower than 0.01% v/v. This MIC was able to inhibit adhesion and biofilm formation in an abiotic surface for both strains assayed.

This study demonstrates that this essential oil from *Lavandula dentata* could be a promising strategy against resistant biofilms from *Candida spp*.

PW487 Bioactive compound produced by endophytic fungi isolated from *Sceletium tortuosum* L.

Madira Coutlyne Manganyi[1], Thierry Regnier[2], Christ-Donald Kaptchouang Tchatchouang[1], Cornelius Carlos Bezuidenhout[3], Collins Njie Ateba[1]

[1]*Department of Microbiology, North-West University-Mafikeng Campus, South Africa*

[2]*Department of Biotechnology and Food Technology, Tshwane University of Technology, South Africa*

[3]*Unit for Environmental Sciences and Management, North-West University-Potchefstroom Campus, South Africa*

Endophytic fungi have the ability to co-exist with their host plants without causing any harm and are beneficial to both the plant and the fungi. They produce bioactive compounds that protect the host plants against attack from insects, pathogens and herbivores. This study determined the antimicrobial properties and identify the metabolites produced by endophytic fungi isolated from *Sceletium tortuosum* L, this report was the first in the world. *Sceletium tortuosum* plants were collected from South Africa. Culturable endophytes were isolated from leaves and roots. Morphological characteristics and internal transcribe spacer (ITS1 and ITS4) were used for identification. Bioactive compounds were detected after fermentation and extraction. Antibacterial properties were determined using the disc diffusion assay against Gram positive and negative bacteria. The chemical compounds were characterized by GC-MS. Sixty fungi belonged to 16 genera which were compared to the GenBank. *Fusarium* species had 37%, followed by *Aspergillus* (25%) and *Penicillium* (7%) species. Phylogenetic analysis revealed three potentially novel species (DR 019 *Fusarium penzigii,* DR 010 *Phomopsis columnaris*, DR 007 *Fusarium oxysporum* f. sp. *lycopersici*). Overall, 15% of fungal extracts displayed narrow spectrum of activity against the bacteria strains. Despite this, none of the fungal extracts inhibited growth of *E. faecalis* (ATCC S1299) and *E. gallinarum* (ATCC 700425) while *B. cereus* (ATCC 10876) was the most susceptible. *Fusarium oxysporum* (GG 008) displayed significant antibacterial activity that were linked to high levels of 5-Hydroxymethylfurfural (HMF) and octadecanoic acid as revealed by GC-MS.

PW488 Assessment of Candida and Aspergillus Species Frequency in Patients with Suspected Fungal Infection

elnaz iraji[1], mehraban falahati[1], shirin farahyar[2], azam fattahi[3], zeinab ghasemi[4], zeinab yasin[5]

[1]Iran University of Medical Sciences , dept. of parasitology and mycology, school of medicine , Tehran, Iran
[2]Iran University of Medical Sciences , Microbial Biotechnology Research center, school of medicine, Tehran, Iran
[3]Tehran university of Medical Sciences , center for research and training in skin disease and leprosy, Tehran, Iran
[4]Iran University of Medical Sciences , Razi hospital, Tehran, Iran
[5]Iran University of Medical Sciences , Firoozgar hospital, Tehran, Iran

Background: During the past several decades, there has been a steady increase in the frequency of opportunistic invasive fungal infections (IFIs) in immunocompromised patients. The high mortality rate of patients suffering IFIs necessitates early diagnosis and target treatment. The symptoms of IFIs are unspecific in the early stages. Moreover, broad spectrum antifungal therapy due to unspecific diagnosis often leads to further complications in patient's condition. Therefore, species differentiation is crucial for target antifungal therapy. Traditional phenotype-based diagnostic methods, which are still considered the gold standards, often have low sensitivity and specificity and are time consuming.

Objectives: Assessment of PCR-RFLP method as a an alternative approach for differentiation between the most significant fungal agents.

Methods: In the present study, a total of 90 clinical specimens including BAL, urine and CSF collected from patients suspected for fungal infection were cultured and colonies were assessed by PCR-RFLP technique, amplifying the ITS1-5.8S-ITS2 region of fungal rDNA genes. Digestion of the PCR products was done using mspI and HpaII restriction enzymes for differentiation of Candida and Aspergillus species, respectively. Using this method, we were able to discriminate among medically important species of Candida and Aspergillus.

Results: Our findings could determine the most frequent Candida and Aspergillus species in clinical specimens which will be fully discussed in the article. Furthermore, it can be stated that PCR-RFLP method is a simple, rapid and cost-effective tool for differentiation among species in clinical laboratories, which can lead to a specific diagnosis of opportunistic fungal agents and target antifungal therapy.

PW489 Biofilm characterization of Fusarium solani keratitis isolate: increased resistance to antifungals and UV light

Itzel Margarita Córdova-Alcántara[1], Diana Laura Venegas-Cortés[1], José Alejandro Hernández Benítez[1], María Ángeles Martínez-Rivera[1], Octavio Pérez Néstor[2], Aida Verónica Rodríguez-Tovar[1]

[1]Instituto Politécnico Nacional, Laboratorio de Micología Médica, Departamento de Microbiología, Escuela Nacional de Ciencias Biológicas, Ciudad de México, Mexico
[2]Probiomed SA de CV, Departamento de Investigación y Desarrollo, Estado de México, Mexico

Background: *Fusarium solani* has drawn phytopathogenic, biotechnological and medical interest. In humans, it's associated with localized infections, such as onychomycosis and keratomycosis, as well as invasive infections in immunocompomised patients. One pathogenicity factor of filamentous fungi is biofilm formation. There is only scarce information about the in vitro mechanism of the formation and composition of *F. solani* biofilm.

Objectives: Describe the stages of formation and composition of the biofilm produced by a clinical keratomycosis isolate of *F. solani,* and assess its susceptibility to different antifungals and ultraviolet light (UV).

Methods: We describe the development of *Fusarium solani* biofilm trough biomass quantification with cristal violet assay and structural analysis with scanning electron microscopy (SEM), its chemical composition by Epfluorescence microscopy (EPM). We assessed the susceptibility of *F. solani* biofilm to antifungal agents with biomass quantification and viability by MTT and to UV light.

Results: We observed five biofilm formation stages using SEM: adherence, germination, hyphal development, maturation and cell detachment (Figure 2) and we propose a model of time-dependent development of it (Figure 9). Using EPM we elucidated the chemical composition of the extracellular matrix: carbohydrates, proteins and extracellular DNA (Figure 4, Figure 5), specific inhibitors for these molecules showed significant biofilm reductions. We observed increased resistance to four antifungals when the biofilm preformation to 24 h, and only the amphotericin B showed activity against the biofilm preformation to 48 h (Figure 8A, Figure 8B). The preformed biofilm at 24 h protected and reduced UV light mortality (Fig 8C).

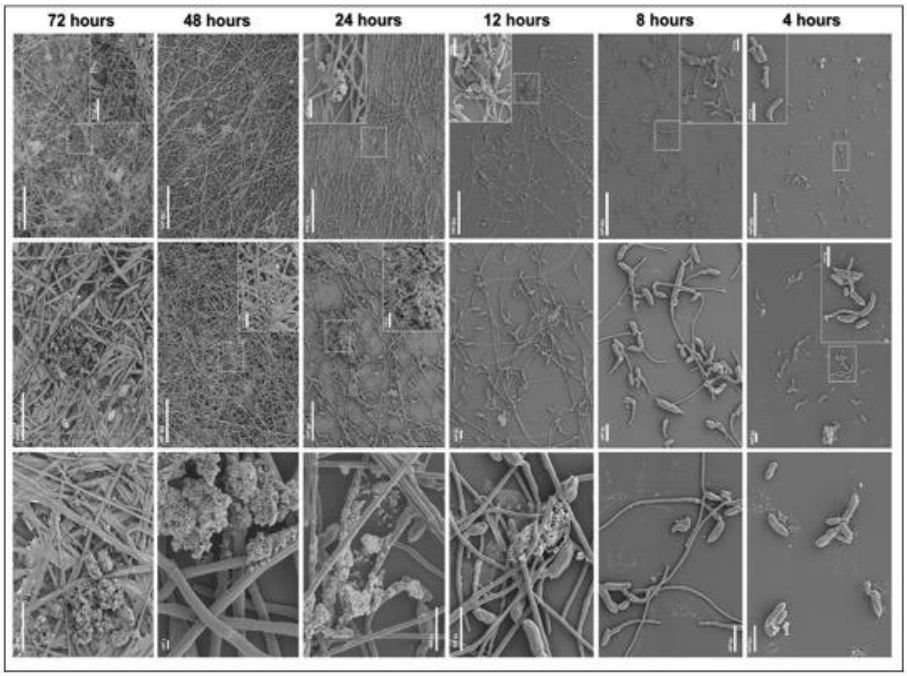


Fig. 2. Stages of *F. solani* biofilm development by SEM. Development of *F. solani* biofilm is displayed by scanning electron microscopy. (4 h), Cell adhesión and cell coaggregation. (8 h), Germination of conidia, elongation, and hyphae development. (12 h), Anastomosis and interweaving of hyphae and beginning of water-channel formation. (24–48 h). Biofilm maturation with well-developed hyphae, anastomosis, water-channel formation, hyphal interlinking, and abundant production of ECM. (72 h), Highly structured mature/old biofilm, dispersed macroconidia and cell detachment. Biofilms are developed in 12-well polystyrene plates incubated at 0–72 h in RPMI medium with a $1x10^6$ conidia/ml final concentration at 28ºC. The white box insert shows a higher magnification detail. Images are representative of three experiments for each one.

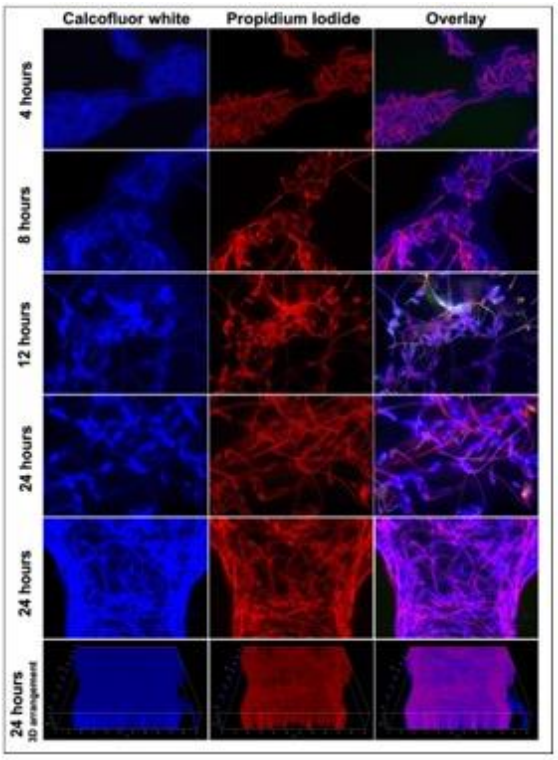


Fig. 4. EPM micrographs of calcofluor white and propidium iodidestained *F. solani* extracellular matrix. The chemical conformation of *F. solani* biofilm is demonstrated by epifluorescence microscopy. *F. solani* biofilms were stained with the fluorochrome calcofluor white (blue color), which binds to chitin, cellulose and glucose residues, and propidium iodide (red color) showed the presence of eDNA surrounding the cell structures in the ECM. All stages of *F. solani* biofilm development are shown from 0 to 24 h. At 24 h is shown a three-dimensional arrangement of the biofilm exposing the chemical component distribution in the ECM. Biofilms are developed in 12-well polystyrene plates incubated for 4–24 h in RPMI medium with a $1x10^6$ conidia/ml final concentration at 28°C, magnification 63X. The extracellular matrix ECM is indicated with a white arrow.

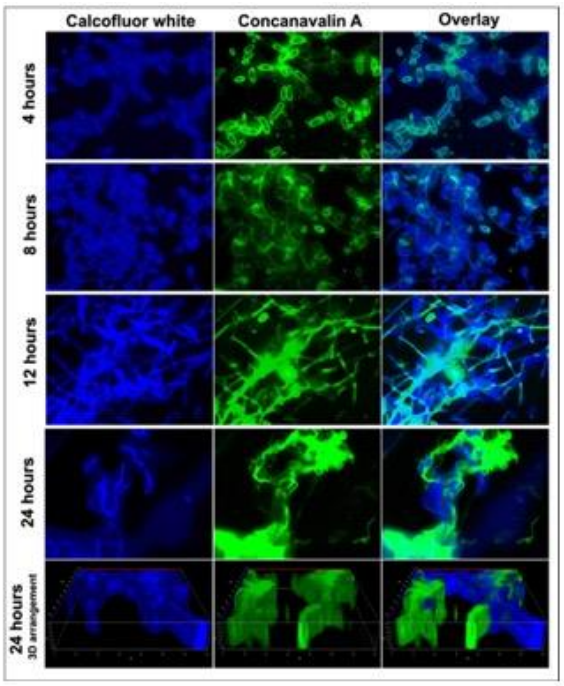


Fig. 5. EPM micrographs of calcofluor white and concanavalin A-stained *F. solani* extracellular matrix. The chemical conformation of *F. solani* biofilm is demonstrated by epifluorescence microscopy. *F. solani* biofilms were stained with the fluorochrome calcofluor white (blue color), which binds to chitin, cellulose and glucose residues, and ConA (green color), which is a lectin with affinity for terminal α-D-mannosyl and α-D-glucosyl residues. All stages of *F. solani* biofilm development are shown from 0 to 24 h. At 24 h is shown a three-dimensional arrangement of the biofilm exposing the chemical component distribution in the ECM. Biofilms are developed in 12-well polystyrene plates incubated for 4–24 h in RPMI medium with a $1x10^6$ conidia/ml final concentration at 28°C, magnification 63X. The extracellular matrix ECM is indicated with a white arrow.

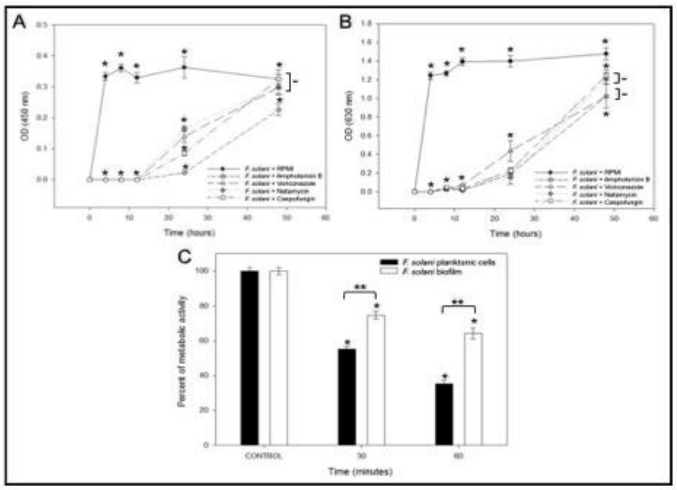


Fig. 8. Susceptibility testing of *F. solani* biofilms to antifungals and UV light. (A) Viability of the *F. solani* biofilm cells exposed to 8 µg/ml amphotericin B (AMB) (gray pentagon), 8 µg/ml voriconazole (VOR) (gray triangle), 8 µg/ml natamycin (NAT) (gray diamond) and 64 µg/ml caspofungin (CAS) (white square); the metabolic activity was measured using the MTT assay on *F. solani* biofilms developed in 96-well polystyrene plates incubated for 0–48 h in RPMI medium with a $1x10^6$ conidia/ml final concentration, *F. solani* biofilm cells were compared with the untreated cells (black circle). (B) Quantification of F. solani biofilm biomass exposed to 8 µg/ml amphotericin B (AMB) (gray pentagon), 8 µg/ml voriconazole (VOR) (gray triangle), 8 µg/ml natamycin (NAT) (gray diamond) and 64 µg/ml caspofungin (CAS) (white square); the biomass was measured using the crystal violet assay on *F. solani* biofilms developed in 96-well polystyrene plates incubated for 0–48 h in RPMI medium with a $1x10^6$ conidia/ml final concentration; *F. solani* biofilm cells were compared with the untreated cells (black circle). (C) The percent metabolic activity of biofilms (white bar) and planktonic (black bar) cells was compared with the unexposed cells after being irradiated by UV light for 30 and 60 min. The biomass was measured using the MTT assay on *F. solani* biofilms developed in 96-well polystyrene plates incubated for 0–48 h in RPMI medium with a $1x10^6$ conidia/ml final concentration. In all cases, comparisons between absorbance levels revealed significant differences [(*), $P < 0.050$] and no significant differences [(-), $P < 0.050$] by the Student-Newman Keuls test performing multiple comparisons of procedures. Values are representative of three experiments with twelve replications for each one.

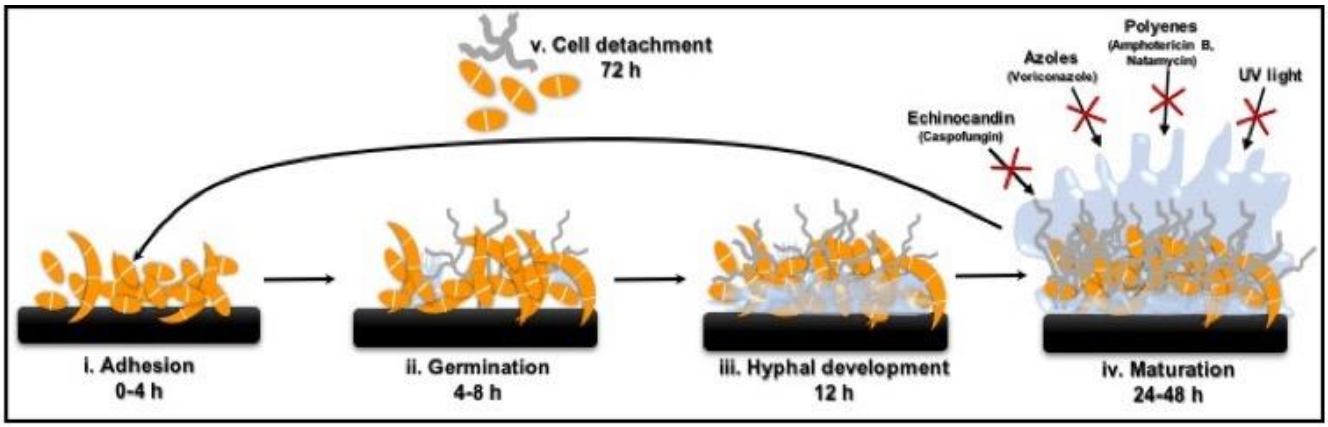


Fig. 9. Schematic representation of the time-dependent development of the biofilm of *F. solani*. The model for biofilm development includes the following stages: i, adhesion (0–4 h); ii, germination (8 h); iii, hyphal development (12 h); iv, maturation (24–48 h); and v, detachment and cell dispersion (72 h).

PW490 toxin gene profiles of bacillus cereus isolated from cooked rice

Yugenraj Navaneethan[1]

[1]*Universiti sains malaysia, food technology department, minden, Malaysia*

Background: The presence of toxin genes in *Bacillus cereus* is responsible for toxin production, leading to gastroenteritis which can be as severe as resulting in death.

Objectives: To determine the distribution of virulence genes (*hblACD*, *nheABC*, *cytK*, *entFM*, and *ces*) in 36 strains of *Bacillus cereus* isolated from cooked rice samples

Methods: Polymerase Chain Reaction (PCR analysis) was used to determine the presence of virulence genes.

Results: A total of 35 strains (97.2%) in this study were devoid of *hblA* and *hblC*. On the contrary, *hblD* was detected in more of the tested strains (30.6%). A total of 10 strains were positive for one gene in *hbl* operon and a single strain harboured all the *hblACD* genes. Similarly, discrepancies were observed for the presence of Nhe-encoding genes with the *nheA*, *nheB* and *nheC* detected in 6 (16.7%), 23 (63.9%), and 24 (66.7%) strains respectively. Most of the strains (69.4%) carried at least one gene in the *nhe* operon with only 3 strains possessing all three *nhe* genes . The detection rates of *cytK* , *entFM* and ces in this study were 58.3%, 77.8% , and 13.9% , respectively. These results indicate that *entFM* are much more broadly distributed among members of *B. cereus*, followed by *nhe* and *cytK* genes as compared to other virulence genes. Moreover, most of the strains (83.33%) were found to harbour at least one of the toxin-encoding genes, and therefore presents increased health risks to consumers.

PW491 Effect of exondation on intertidal bivalve digestive microbiota

Clément Offret [1], Sauvann Dubois[1], Kevin Château[1], Olivier Gauthier[1], Adeline Bidault[1], Philippe Miner[1], Bruno Petton[1], Hélène Hegaret[1], Caroline Fabioux[1], Fabrice Pernet[1], Charlotte Corporeau[1], Christine Paillard[1], Gwenaelle Le Blay[1]

[1]*Laboratoire des Sciences de l'Environnement MARin (LEMAR - UMR 6539)- CNRS UBO IRD IFREMER, Institut Universitaire Européen de la Mer, Plouzané, France*

Background: Microbiota, including digestive microbiota, provide a wide range of beneficial effects on their host physiology and resistance to infections. Marine intertidal bivalves face important habitat variations due to tidal cycles while they are confronted with anthropogenic forcing and resurgence of pathogens. In this context, it is necessary to know which factors impact their health and their ability to acclimatize. Currently few is known about bivalve microbiota and nothing about exondation effect on their microbiota, although it is known that tidal cycles play a key role in their physiology.

Objectives: The main objective of this study was to characterize exondation effect on the digestive microbiota of *Ruditapes philippinarum*, and *Crassostera gigas* in the field.

Methods: Bivalves were implanted at three exondation levels (20, 56 and 80 % of time) from October 2017 to February 2018 in the Bay of Brest, France. In February 2018, we sampled water, sediments and animals (60 by levels), and half of the bivalves were placed in depuration for 15 days in controlled conditions in laboratory. Then, digestive-gland DNA were extracted and analyzed by 16S rRNA gene amplicon sequencing (Illumina) to determine their microbiota composition in the field and after depuration.

Results: First results show a strong modification of the digestive gland microbiota after 4 months of implantation, associated with a significant effect of the exondation, especially for *Ruditapes philippinarum*. This effect was still observed after depuration. The exondation response was mediated by changes in the OTUs while little changes were observed at higher taxonomic levels.

PW492 metagenomic profiling of bacterial genes indicative for nutrient cycling and plant-beneficial functions in termite mound soils

Ben Jesuorsemwen Enagbonma[1], olubukola oluranti babalola[2]

[1]*north-west university, microbiology, Mmabatho, Mafikeng, North-West province, South Africa*
[2]*north west university - mafikeng, microbiology, mmabatho, South Africa*

Background: Termite mound soils host a variety of bacteria which are capable of contributing to ecological services. However, information regarding their functional genes are very poor.

Objectives: In this study our aim was to investigate the relative abundance and the diversity of the various bacteria genes indicative for carbon fixation, nitrogen and sulfur cycling and plant-beneficial functions present in termite mound soils and to see if they are similar or different from those genes present in their surrounding soils.

Methods: 0.25 g soil samples were used for whole DNA extraction using the PowerSoil DNA isolation kit. Afterward**, the extracted DNA was sequenced using the** shotgun metagenome sequencing approach. The raw sequences of each of the metagenomes were uploaded to the metagenomics rapid annotation (MG-RAST) server for downstream analysis.

Results: Our result showed that *Proteobacteria* and *Actinobacteria* were most abundant in termite mound soils and their surrounding soil samples respectively with carbohydrate metabolism observed as the dominant functional category in all soil samples. Furthermore, genes related to carbon fixation, nitrogen cycling, sulphur cycling and plant beneficial functions were observed in both soil sample. Statistical evaluation showed that the functional gene abundance in termite mound soils only differ significantly from that of the surrounding soils in carbon fixation, pyrroloquinoline quinon encoding genes, 1-aminocyclopropane-1-carboxylate deaminase gene, acetoine genes, and 2, 3-butanediol genes. Thus, this study extends our awareness of the bacterial composition and functional genes present in termite mound soils, and their role in nutrient cycling and plant growth enhancement.

PW493 Prevalence and Virulence Determinants of Staphylococcus aureus Isolated from Recreational Waters and Beach Sand in Eastern Cape Province, South Africa

Emmanuel Akanbi[1], Anthony Otigbu[2], Henry Njom[3], Justin Fri[2]

[1]*University of Fort Hare, Biochemistry and Microbiology, Alice, South Africa*
[1]*University of Fort Hare, Biochemistry and Microbiology, Alice, South Africa*
[3]*Agricultural Research Council, Potchefstroom, South Africa*

Background: *Staphylococcus aureus* is a Gram-positive bacterium predominantly found in human skin and nasal passages with 20 to 40% of the population carrying this organism. it is a perilous human pathogen associated with both nosocomial and community-acquired infections and is increasingly becoming virulent.

Objectives: This study determined the occurrence and virulence determinants of *S. aureus* in beach water and intertidal beach sand.

Methods: *S. aureus* was isolated from 249 beach sand and water samples using standard microbiological methods and subsequently screened for virulent genes. Virulent genes encoding intracellular adhesion (*ica*A), enterotoxin (*sea*A) and cytolytic toxins (PVL) were screened.

Results: From a total of 249 samples, (n=178) was beach water and (n=67) was sand samples. Thirty isolates 30/245 (12%) were confirmed as *S. aureus* of which 22/178 (12.4%) of isolates were from seawater and 8/67 (11.9%) were from marine sand. Of the 22 confirmed *S. aureus* isolates from seawater, 6 isolates each were from Middle beach and Eastern beach, 5 isolates from Nahoon beach, 2 each from Kariega beach and East beach and 1 isolate from West beach. Of the 8 confirmed *S. aureus* isolates from sand, 4 were from Middle beach and 2 each were from Kariega beach respectively. The PVL, *ica*A and *sea*A genes encoding virulent determinants were detected in 50%, 20% and 13% respectively in the confirmed isolates respectively. Results emphasize the need for the implementation of better control measures to reduce the occurrence of virulent *S. aureus* strains in recreational waters.

PW494 Intestinal parasitic infections among prison inmates in Kathmandu Nepal

Prasha Shrestha[1], Dhiraj Shrestha[1], Ganesh Rai[1], Kul Raj Rai[1;2], Shiba Kumar Rai[1]

[1]*Shi-Gan International College of Science and Technology, Department of Microbiology, Kathmandu, Nepal*
[2]*University of Chinese Academy of Science, Institute of Microbiology, Beijing, China*

Background: Prison inmates are at high risk of intestinal parasitic infections (IPIs).

Objectives: The objective was to find the IPIs among the inmates of the Central Jail in Kathmandu, Nepal.

Methods: Morning stool samples from 400 inmates (M=282 and F=118) were collected in a clean, dry and wide-mouthed plastic container. The samples were transported to the research laboratory of Shi-Gan International College of Science and Technology and were fixed using 10% formal saline. Then, samples were processed by formal ether sedimentation concentration technique and were observed microscopically by direct-smear technique.

Results: Only 6% (24/400) samples were positive for intestinal parasites, with a gender ratio (M: F) 1.7:1. But, co-parasitism was not observed. IPIs were higher among 21-40 years age-group, 3.5% (14/262). Similarly, IPIs were higher among '*Dalits*' ethnic group, 21.1% (4/19). As compared to helminths, more protozoans, 62.5% (15/24), were observed. *Giardia lamblia,* 41.67% (10/24), was the most common protozoans while *Trichuris trichiura*, 25.0% (6/24), was the most common helminths.

PW495 Insights on aquatic microbial communities of Indian Sundarbans: ecological indicators in a wildlife reserver

Paltu Dhal[1]

[1]*Kolkata, Department of Life Science and Biotechnology, Jadavpur University, Kolkata, India*

Background: Pollution has a strong impact on water quality and ecological health of mangrove areas of Indian Sundarbans. However, microbial communities of estuarine water in this region and their responses to water quality were seldom studied

Objectives: This study attempted to report bacterial and archaeal communities of water from Matla River and Thakuran River of Maipith coastal areas more accurately.

Methods: The accurate and well-resolved picture of bacterial and archaeal communities simultaneously of marine water in Sundarbans mangrove using next-generation amplicon sequencing of the 16S rRNA gene using recently developed 515F-Y/926R primer set

Results: Our investigation indicates that the water with a lower nutrient load of a small Island of Sundarban Mangroves (ISM), was dominated by halophilic marine bacteria from families Flavobacteriaceae and OM1 clade. At higher eutrophic conditions, changes in bacterial communities of Open Marine Water (OMW) were detected, where some of the oligotrophic marine hydrocarbons degrading bacteria of the families Oceanospirillaceae and Spongiibacteraceae were dominated. Rhodobacteraceae alone represented 18% of the total community of both sites being the most abundant bacterial family. However, not much change was observed among archaeal composition. Redundancy analysis indicates a combination of total nitrogen and dissolved inorganic nitrogen for OMW and for ISM salinity and total nitrogen were responsible for explaining changes in their respective microbial community composition. Systematic approaches with more samples for evaluating the effect of environmental pollutions on mangrove microbial communities are recommended

PW496 Degradation of branched-chain amino acids in the presence of thiosulfate and external fatty acids in Thermoanaerobacter species

Johann Orlygsson[1]

[1]*University of Akureyri, Faculty of Natural Resource Science, Akureyri, Iceland*

Background: Physiology of thermphilic anaerobic amino acid degrading bacteria. The presentation will cover how amino acids are degraded anaerobically with special emphasis on branched-chain amino acid coversion to their corresponding fatty acids and alcohols

Objectives: The main objectives of the study is to gain deeper understanding of the role of electron scavenging systems for the degradation of branched-chain amino acids. Two systems are investigated; addition of thiosulfate as an electron acceptor and co-cultivating the amino acid degrading thermophile in a co-culture wiht a hydrogenotrophic methanogen

Methods: Anaerobic cultivation using the Hungate technique, Analysis of substrates and end products by GC, HPLC, NMR. Enzymatic assays using spectorphotometer

Results: The main outcome of the study is that bacteria belonging in the genus *Thermoanaerobacter* degrade the branched-chain amino acids only in the presence of electron scavenging systems. When thiosulfate is added the bacteria degrade the branched-chain amino acids to a mixtue of the acids and alcohols but when the amino acid degrading bacteria is co-cultivated with a hydrogenotrophic methanogen only the acids are produced. Finally, NMR studies show that these bacteria can, under certain conditions, convert fatty acids to their corresponding alcohols

PW497 Molecular relationship between the resistance to quaternary ammonium compounds (QAC) and antibiotic resistance in coagulase-negative staphylococci(CoNS) isolated from ready-to-eat food

Wioleta Chajęcka-Wierzchowska[1], Anna Zadernowska[1], Urszula Zarzecka[1]

[1]*University of Warmia and Mazury in Olsztyn, Chair of Industrial and Food Microbiology, Olsztyn, Poland*

Background: The food and cathering industries are frequently using disinfectants based on quaternary ammonium compounds (QAC) to eradicate and to prevent the spread of unwanted microorganisms. This may result in residual sublethal concentration of QAC in the environment, which, in turn, may lead to a selective pressure for retention of resistance genes encoding resistance to both disinfectant and antibiotics among microorganisms.

Objectives: We aimed to investigate the prevalence of genes encoding QACs and antibiotics resistance in CoNS isolated from ready-to-eat food from bars and restaurants.

Methods: Minimal inhibitory concentration (MIC) of benzalkonium chloride (BC) and phenotypic antibiotic resistance in 112 CoNS strains were investigated. In the resistant strains the presence of genes encoding resistance to methicillin (*mec*A), aminoglycosides [*aac*(6')/*aph* (2"), *aph*(3)-III, *ant* (4')-*Ia*)], tetracyclines (*tet*M, *tet*L, *tet*K), macrolides (*erm*A, *erm*B, *erm*C, *msr*, *mef*A/E) and QAC (*qac*A/*qac*B, *qac*C) were determined.

Results Multi-resistant strains towards antibiotics and QAC within CoNS from food were found. The resistant genes to QAC were detected in 56,3% of CoNS isolates with reduced susceptibility to BC. The most frequent detected genes were *qac*A/B in 34% of isolates. There was an association between the presence of *qac* genes and resistance to different antibiotics which may be attributed to presence of both groups of the genes on the same plasmid or due to selection of resistant strains. Our molecular studies demonstrated that some food-related staphylococci from different sources harbored both the *qac*B, *mec*A, *ermB* and/or *aac*A-*aph*D genes on plasmid suggesting genetic linkage between disinfectant and antibiotic determinants.

PW498 Unveiling the bacterial community in leachates from solid waste at a major landfill in Lagos, Nigeria

Adewale Ogunyemi [1], Titilola Samuel[2], Olukayode Amund[1], Mathew Ilori[1]

[1]*UNIVERSITY OF LAGOS, MICROBIOLOGY, LAGOS, Nigeria*
[2]*UNIVERSITY OF LAGOS, BIOCHEMISTRY, LAGOS, Nigeria*

Background: The increased rates of solid waste generation in major cities of Nigeria and associated risk of environmental pollution have necessitated a better understanding of the role of bacteria in waste conversion of leachate and the need for better waste management in the country.

Objectives: This study was conducted to determine the microbial community structure at a major landfill in Lagos, using a metagenomic approach.

Methods: Olushosun landfill was selected by simple random technique. The samples were pooled in the laboratory and processed for metagenomic DNA extraction using the Zymo kit. This was followed by 16S rDNA amplification by PCR and amplicon sequencing using Illumina sequencing approach at Inqaba Biotech. (South Africa). The BLAST-based data analysis was performed using an Inqaba in-house developed data analysis pipeline.

Results: Twenty megabyte (20Mb) of data (2x300bp long paired end reads) was produced from the pooled sample with bacteria constituting 99.87% of the total read counts (i.e. 74766 read count). The phyla of bacteria detected in decreasing order of abundance were *Acidobacteria* (14.65%) followed by *Planctomycetes* (7.15%), *Proteobacteria* ((3.28%), *Chloroflex*i (1.41%), *Actinobacteria* (0.91%)*, Verrucomicrobia* (0.30%) and *Gemmatimonadetes* (0.20%), Other bacterial phyla were *Firmicutes* (0.17%), *Nitrospira* (0.17%), *Bacteroidetes* (0.06%), *Chlamydiae* (0.03)*, Deferribacteres* (0.01%), *Cyanobacteria* (0.01%) and *Tracheophyta* (0.01%). The *Euryarchaeota phylum was not detected and* 71.64% of the bacterial communities had unclear phylum affiliation.

Conclusions: This study reveals a high diversity of bacteria community in a major landfill in Lagos with potential for bioprospecting for better waste management in the nearest future.