

Abstractbook

from 2024-05-09 with 121 Abstracts





Detailed Program



Tuesday, May 14th, 2024

 10:00 - 10:20 WELCOME 10:20 - 10:55 OPENING LECTURE: Franciska De Vries (University of Amsterdam, NL) Understanding soil microbiome response to climate change SESSION 1 Microbiomes and the fertilizer crisis 10:55 - 11:45 Chairs: Carolin Schneider, Trevor Charles 10:55 - 11:20 Trevor Charles (University of Waterloo, CA) Enrichment for and selection of novel bacterial isolates with potential as biofertilizers for soil-less agriculture 11:20 - 11:35 Marco Giovannetti (University of Torino, IT) Lactuca sativa genetic variation shapes the plant phenotypic and metabolic response to a soil microbial inoculum 11:35 - 11:45 SCIENCE FLASHES: Marta Acin (Biome Makers, ES) Harnessing biostimulants and data-driven microbiome analysis to address the fertilizer crisis: A sustainable approach Annamaria Bevivino (ENEA, Italian National Agency for New Technologies, Energy and Sustainable Economic Development, IT) Potential of microbiome-based solution as green biofertilizer for sustainable maize 	
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productivity	
11:45 - 12:15 COFFEE BREAK	
SESSION 1MICROBIOMES AND THE FERTILIZER CRISIS12:15 - 12:55Chairs: Carolin Schneider, Trevor Charles	_
12:15 - 12:40 Carolin Schneider (Inoq GmbH, DE) What can microbial plant biostimulants do for our food quality: The mycorrhiza- tomato story	
12:40 - 12:55 Sara Borin (University of Milan, IT) The microbiome associated to the weed <i>Panicum</i> sp. exploited as self-produced sustainable biofertilizer for staple crops in Sri Lanka	
12:55 - 14:30 LUNCH BREAK & POSTER SESSION I	_

- SESSION 2 MICROBIOMES MITIGATING BIOTIC AND ABIOTIC STRESS
- 14:30 16:15 Chairs: Jesús Mercado-Blanco, Simone Gatzke
- 14:30 14:55 Jesús Mercado-Blanco (Estación Experimental del Zaidín (CSIC), ES) What the olive tree holobiont needs to confront a soil-borne fungal pathogen (and how to help it)
- 14:55 15:20 Simone Gatzke (Forschungszentrum Jülich GmbH, DE) The role of phenotyping facilities to analyze plant-microbial interactions and their access possibilities across Europe
- 15:20 15:35 **Joseph Selvin (Pondicherry University, IN)** The role of microbiome shift on the emergence of climate resilient coral species
- 15:35 15:50 **Eleonora Rolli (University of Milan, IT)** Plants 'cry-for-help' in presence of the xenobiotic polychlorinated biphenyls
- 15:50 16:05 Andrea Visca (ENEA, IT) Unveiling the microbial players: Metagenomic insights into olive varieties' response to drought
- 16:05 16:15 SCIENCE FLASHES:
 Sonia Mazzarino (University of Turin, IT)
 Isolation and selection of plant-associated microbes for the formulation of new inocula to be used in sustainable agriculture

Sara Berzuini (University of Bologna, IT)

Effect of different beneficial microorganisms in the growth promotion of camelina (*Camelina sativa* L. Crantz)

- 16:15 16:40 **COFFEE BREAK**
- WORKSHOP WORKSHOP THE MICROBIOMESUPPORT ASSOCIATION
- 16:40 18:00 Workshop organizers: The Association Founding Members
- 18:00 20:00 POSTER SESSION I & NETWORKING (WINE & CHEESE)

Wednesday, May 15th, 2024

09:00 - 09:25 Michael Schloter (Technical University of Munich, DE) Age matters: Exploring differential effects of antimicrobial treatment on gut microbiota of adult and juvenile brown trouts and non-target organisms of the aquatic ecosystem 09:25 - 09:50 Sharon Huws (Queen's University Belfast, GB) Microbiomes: Friend or foe in the battle against antimicrobial resistance 09:50 - 10:05 Stéphane Chaillou (INRAE, FR) Longitudinal study of chicken microbiomes from egg to meat: limpact of farming practices 10:05 - 10:20 Narciso M. Quijada (University of Salamanca, ES) The resistome investigation in foods and their processing environments from 113 European companies reveals high connection with mobile genetic elements and ESKAPEE bacteria 10:20- 10:30 SCIENCE FLASHES: Tea Movsesijan (Austrian Competence Centre for Feed and Food Quality, Safety & Innovation (FFoQSI), AT) Deciphering microbial dynamics: Investigating the impact of a novel supplement on canine gut microbiota 10:30 - 11:00 COFFEE BREAK SESSION 4 MICROBIOMES AND (GHG, MANURE) EMISSIONS 11:00 - 11:25 Hauke Smidt (Wageningen University & Research, NL) 11:00 - 11:25 Hauke Smidt (Mageningen University & Research, NL) 11:00 - 11:25 Hauke Smidt (ROBIOMES AND EPIDEMICS 11:25 - 11:50 Fool SYSTEMS MICROBIOMES AND EPIDEMICS	SESSION 3 09:00 - 10:30	ANIMAL WELFARE, ANTIBIOTIC RESISTANCE, AND ROBUSTNESS Chairs: Michael Schloter, Sharon Huws	
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12:30 - 14:00 LUNCH BREAK & POSTER SESSION II

SESSION 5 FOOD SYSTEMS MICROBIOMES AND EPIDEMICS

- 14:00 15:10 Chairs: Marco Candela, Søren Sørensen
- 14:00 14:25 Søren Sørensen (Department of Biology University of Copenhagen, DK) Exploring antimicrobial resistance and plasmid diversity in the early life gut microbiome
- 14:25 14:40Sophie Hautefeuille (INRAE, FR)
Understanding the association between the bacterial communities of broiler meat
and *Campylobacter* for enhanced food safety
- 14:40 14:55Cristian Botta (University of Turin, IT)The pros and cons of tracking the microbial contamination in infant food processing
chain through amplicon sequencing and metagenomics
- 14:55 15:10 SCIENCE FLASHES:

Fulvia Troja (University of Bologna, IT)

Use of shotgun metagenomic for the analysis of shellfish virome: Comparative assessment of the performances of three capture enrichment kits

Valentina Riva (University of Milan, IT)

Deciphering the dynamics of antibiotic resistance transfer through natural transformation in bacterial communities

Elisabetta Chiarini (University of Turin, IT)

Meta-taxonomic analysis of poultry and slaughterhouse microbiota: A comprehensive examination of resident microbial communities

15:10 - 15:20 HEALTH BREAK

WORKSHOP NEW FOODS/DIETS AND GUT MICROBIOME HEALTH

15:20 - 16:20 Workshop organizers: Yolanda Sanz (CSIC, ES), Emmanuelle Maguin (INRAE, FR)

The session will update scientific evidence on the relationship between long-term dietary patterns, the gut microbiome and human health and discuss how shifts in dietary habits related to food systems transformation could impact the gut microbiome and our health trajectory in the future.

16:20 - 16:50 **COFFEE BREAK**

SESSION 6 MICROBIOME DIVERSITY AND FOOD QUALITY

- 16:50 18:00 Chairs: Martin Wagner, Inga Sarand
- 16:50 17:15 Inga Sarand (Tallinn University of Technology, EE) Interplay between microbiome and storage conditions in spoilage mitigation

17:15 - 17:30 Hugo Roume (Lesaffre, FR) Forecasting sourdough aromatic profiles by integrated multi-omics data into a community-wide metabolic network

17:30 - 17:45 **Davide Buzzanca (Università degli Studi di Torino, IT)** PDO and non-PDO Roccaverano cheeses: Exploring microbiome diversity and correlations

17:45 - 18:00 **Eva Maria Molin (AIT Austrian Institute of Technology Gmb, AT)** From science to practice: Bio-preservation for storage improvement – a sugar beet case study

Thursday, May 16th, 2024

SESSION 6	MICROBIOME DIVERSITY AND FOOD QUALITY
09:00 - 09:40	Chairs: Martin Wagner, Inga Sarand

- 09:00 09:25 Martin Wagner (University for Veterinary Medicine, AT) Contamination maps as a tool to improve and secure food production in a farm-tofork strategy
- 09:25 09:40 SCIENCE FLASHES:

Francesca Cristetti (University of Turin, IT)

Exploration of microbial ecology as a quality marker through its linkage to the geographical origin of spontaneously fermented food matrices: The case of wine, green coffee beans, and cocoa beans.

Franz-Ferdinand Roch (University of Veterinary Medicine, Vienna, AT) Dynamic interactions between bacterial and fungal communities on vacuum

packaged beef: A comprehensive longitudinal microbiome analysis

Aashish Jha (New York University Abu Dhabi, AE)

Substrate drives densely connected microbial community assembly across diverse traditional fermented foods.

- SESSION 7 THE EDIBLE MICROBIOME
- 09:40 10:50 Chairs: Tanja Kostic, Gabriele Berg
- 09:40 10:05 **Tanja Kostic (AIT Austrian Institute of Technology, AT)** The edible microbiome: Rethinking the concept of "You are what you eat"
- 10:05 10:30 Gabriele Berg (TU Graz, AT) The edible microbiome and human (gut) health
- 10:30 10:45Giacomo Mantegazza (University of Milan, IT)Microbes on the menu: Investigating rocket salad as a vehicle for microorganisms
for the human intestinal ecosystem

10:45 - 10:50 SCIENCE FLASH: Matevž Zlatnar (Graz University of Technology, AT) Unraveling the fermented olives microbiome: Bacterial diversity, function and its importance for human health

- 10:50 11:20 **COFFEE BREAK**
- SESSION 8 MICROBIOMES FOR IMPROVING HEALTH AND WELL-BEING
- 11:20 13:10 Chairs: Paul D. Cotter, Celia Herrera-Rincon
- 11:20 11:45Celia Herrera-Rincon (Complutense University of Madrid, ES)From the microbiome to the electrome: Implications on the gut-brain axis
- 11:45 12:10 Paul D. Cotter (Teagasc, IE) Harnessing microbiome data to create the next generation of fermented foods

12:10 - 12:25 Elisa Quarta (Universidad Complutense de Madrid, ES) Nutritional iron deficiency effects on microbiota-gut-brain axis

12:25 - 12:40 Mathias Richard (INRAE, FR)

Fungi used in food processes with potential probiotic effects on gut inflammation

12:40 - 12:55 Jekaterina Kazantseva (TFTAK, EE) Intervention study on the effect of fermented vegetable consumption on gut microbiota for three various focus groups

12:55 - 13:10 SCIENCE FLASHES:

Gianfranco Picone (University of Bologna, IT)

The application of High-Resolution Nuclear Magnetic Resonance (HR NMR) in metabolomic analyses of meconium and stool in newborns - the MABEL project: A preliminary study

Nicola Mangieri (University of Milan, IT)

Role of fermented oat drink and fermented milk compared to freeze-dried cells in the survival of the probiotic strain *Lacticaseibacillus rhamnosus* CRL 1505 in human gastrointestinal transit

Agapi Doulgeraki (Aristotle University of Thessaloniki, GR)

Impact of an 8 weeks intervention with orange juice enriched with probiotics and vitamin D on the diet of volunteers at high cardiometabolic risk

13:10 - 14:40 LUNCH BREAK & POSTER SESSION II

SESSION 10 CIRCULAR FOOD SYSTEMS FOR MICROBIOMES IMPROVING ANIMAL, HUMAN AND 14:40 - 16:10 ENVIRONMENTAL HEALTH

Chairs: Lene Lange, Dennis Sandris Nielsen

- 14:40 15:05 Lene Lange (LL-BioEconomy, DK) Circular food systems for microbiomes improving animal, human and environmental health
- 15:05 15:30 Dennis Sandris Nielsen (University of Copenhagen, DK) Use of microbes to produce more climate friendly food and feed
- 15:30 15:45 Anna Clocchiatti (Universiteit van Amsterdam, NL) Exploring soil biome complexity: regional impacts of land management and soil degradation
- 15:45 16:00 Enrique Cubas-Cano (ITENE, ES) TRIBIOME: Developing new circular tools for wheat microbiome modulation as an innovative contribution for a sustainable, healthy, and resilient food production system

16:00 - 16:10 SCIENCE FLASHES: Soumya Sahai Saxena (Indian Institute of Technology Delhi, IN) Utilizing indigenous agricultural soil microbiota for the management of *Listeria* monocytogenes in Indian arable land

Nelson Mota de Carvalho (Escola Superior de Biotecnologia da Universidade Católica Portuguesa, PT)

Supplementation of agro-industrial by-products in animal feed towards well-fare and sustainability: Case study of sugarcane bagasse lignin

17:00 - 23:00 SOCIAL PROGRAMME

Friday, May 17th, 2024

SESSION 9	MICROBIOMES FOR PLANT FERMENTATION
09:00 - 10:15	Chairs: Christèle Humblot, Raffaella Di Cagno

09:00 - 09:25 Christèle Humblot (IRD, FR) Plant based fermented foods for healthier diet, addressing nutritional challenges through tradition and innovation

- 09:25 09:50 Raffaella Di Cagno (Free University of Bolzano, IT) Exploring the metabolic labyrinth of lactic acid bacteria in the microbiome of fermented plant foods: Toward a shift in perspective from the individual to the global
- 09:50 10:05 Maxime Borry (Max Planck Institute for Evolutionary Anthropology, DE) Ancient DNA insights into 7th century Tibetan barley beer brewing from Samdzong, Upper Mustang, Nepal

10:05 - 10:15 SCIENCE FLASHES: Romane Junker (INRAE, FR) Understanding taxonomic diversity and functional signatures of fermented vegetables microbiome

Afolake Olanbiwoninu (Ajayi Crowther University, NG) Metagenomic analyses revealed bacterial communities and diversities in fermented African locust beans

- 10:15 10:45 **COFFEE BREAK**
- SESSION 11 PRESERVATION OF FOOD SYSTEMS MICROBIOMES
- 10:45 11:45 *Chairs:* Christina Warinner, Matthew Ryan
- 10:45 11:05 Matthew Ryan (CABI, GB) Underpinning the food systems microbiome through optimised preservation approaches
- 11:05 11:30 Christina Warinner (Harvard University, US) The milk paradox
- 11:30 11:45 Federico Sbarra (ENEA, IT) Holistic approach to study soil rhizosphere microbiomes and preservation strategies

11:45 - 12:00 **HEALTH BREAK**

WORKSHOP FOOD SYSTEMS KNOWLEDGE AND TECHNOLOGY GAPS

12:00 - 13:00 **Workshop organizers:** Antton Alberdi (University of Copenhagen, DK), Cedric C. Laczny (University of Luxembourg, LU)

The session will discuss key challenges in microbiome research, addressing biological resolution, spatial resolution, and system control, with corresponding technological innovations, including multi-omics analysis, micro-scale metagenomics, and gut-ona-chip models. This will be followed by an extended dialogue on the application of these techniques in food sciences, featuring experts from diverse fields and technical backgrounds.

- 13:00 14:00 LUNCH BREAK
- SESSION 12 CONNECTIVITY OF MICROBIOMES IN THE FOOD SYSTEM
- 14:00 15:05 Chairs: Danilo Ercolini, Angela Sessitsch
- 14:00 14:25Angela Sessitsch (AIT Austrian Institute of Technology, AT)Microbiome interconnectedness throughout environments with major
consequences for healthy people and a healthy planet
- 14:25 14:50 Danilo Ercolini (University of Naples Federico II, IT) Extensive microbiome mapping in the food industry as a strategy to identify functional landscapes for bioprotection and food waste reduction
- 14:50 15:05 Chiara Traina (University of Turin, IT) Microbial communities of Taggiasca fermented olives and olive tree: Preliminary study combining culture-dependent and independent approaches
- 15:05 15:35 **CLOSING LECTURE:**
 - Karel Callens (FAO, IT)

Microbiomes at the crossroads: Pathways for sustainable food systems and the SDGs

15:35 - 16:00 AWARDS & CLOSING

Poster Table



Poster #	Poster Session 1: Microbiomes and the fertilizer crisis	Title
PS1-S1- PP01	Lorenzo Vergani	Study of the application of bio-based materials for the delivery of plant-growth promoting bacteria
PS1-S1- PP02	Birgit Wassermann	Reduced nitrogen fertilization enriches nitrogen-fixing bacteria in the <i>Brassica napus</i> seed microbiome across successive generations
PS1-S1- PP03	Pedro Mondaca	Effects of sustainable agricultural practices on soil microbial diversity, composition, and functions
PS1-S1- PP04	Gabriele Bellotti	Harnessing nitrogen fixation: Advances in diazotrophic bacteria engineering for sustainable crop production
PS1-S1- PP05	Federica Zanetti	Exploring the impact of intercropping camelina-pea on soil and rhizospheric microbiome dynamics and crop productivity
PS1-S1- SF01	Marta Acin	Harnessing biostimulants and data-driven microbiome analysis to address the fertilizer crisis: A sustainable approach
PS1-S1- SF02	Annamaria Bevivino	Potential of microbiome-based solution as green biofertilizer for sustainable maize productivity
Poster #	Poster Session 1: Microbiomes mitigating biotic and abiotic stress	Title
Poster # PS1-S2- PP01	Poster Session 1: Microbiomes mitigating biotic and abiotic stress Francesca Mapelli	Title Bacterial plant probiotics modulate the endophytic community structure in grapevine micropropagated plants
Poster # PS1-S2- PP01 PS1-S2- PP02	Poster Session 1: Microbiomes mitigating biotic and abiotic stress Francesca Mapelli Giorgia Siviero	Title Bacterial plant probiotics modulate the endophytic community structure in grapevine micropropagated plants Evaluation of the association of a PGPB strain RCA25 with Introgression lines (ILs) of <i>Oryza rufipogon</i> X <i>Oryza sativa cv Vialone Nano</i>
Poster # PS1-S2- PP01 PS1-S2- PP02 PS1-S2- PP03	Poster Session 1: Microbiomes mitigating biotic and abiotic stress Francesca Mapelli Giorgia Siviero Lucia Foresto	Title Bacterial plant probiotics modulate the endophytic community structure in grapevine micropropagated plants Evaluation of the association of a PGPB strain RCA25 with Introgression lines (ILs) of Oryza rufipogon X Oryza sativa cv Vialone Nano TRIBIOME project: The influence of abiotic stressors on the wheat microbiome
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Poster #	Poster Session 1: Animal welfare, antibiotic resistance, and robustness	Title
PS1-S3- PP01	Primož Treven	Metagenomic insight into microbiome and resistome of probiotics, starter cultures, and cheeses
PS1-S3- PP02	Daniela Leuzzi	Detection, spread, and transmission of antimicrobial resistant bacteria from swine farms to the surrounding environment
PS1-S3- PP03	Federico Correa	EU-CIRCLES project: Evolution of pig microbiota and health under different farming conditions
PS1-S3- PP04	Emmanuelle Helloin	Creation of an "Egg-to-Meat" biobank of microbiota collected from broiler chickens raised with or without outdoor access
PS1-S3- SF01	Tea Movsesijan	Deciphering microbial dynamics and antimicrobial resistance spread in beef production
PS1-S3- SF02	David Atuahene	Unravelling gut dynamics: Investigating the impact of a novel supplement on canine gut microbiota
Poster #	Poster Session 1: Food systems microbiomes and epidemics	Title
PS1-S5- PP01	Lauren Alteio	Eco-evolutionary factors supporting microbial persistence in food processing environments
PS1-S5- PP02	Yitagele T. Mekonnen	Shotgun metagenomic investigations to detect zoonotic agents in the food system: A CIRCLES project showcase
PS1-S5- PP03	Jessica A Gray	Fresh produce sampling method development for reducing host DNA
PS1-S5- PP04	Indra Bergval	Longitudinal analysis of microbial diversity and dynamics during storage of chicken products: Towards early warning of risks posed by foodborne pathogens
PS1-S5- PP05	Rine Reuben	Antimicrobial and probiotic properties of potential bacteriocinogenic lactic acid bacteria from African traditional foods of animal and non-animal origins
PS1-S5- PP06	Rocio Olmo	Microbiome investigation in Austrian hard-cheese production reveals taxonomic and functional networks between surfaces and food products at different stages of production

PS1-S5-

PP08

Juliette Poujol de Molliens

Microbiota dynamics and differences in growth patterns among *Listeria monocytogenes* and *Listeria innocua* strains in UHT (Ultra High-Temperature) and raw milk at 4°C

PS1-S5- PP09	Antonia Corvino	Real-time volatilomics of food microbiome: The potential of providing temporal dimension in multi-omics studies
PS1-S5- SF01	Fulvia Troja	Use of shotgun metagenomic for the analysis of shellfish virome: Comparative assessment of the performances of three capture enrichment kits
PS1-S5- SF02	Valentina Riva	Deciphering the dynamics of antibiotic resistance transfer through natural transformation in bacterial communities
PS1-S5- SF03	Elisabetta Chiarini	Meta-taxonomic analysis of poultry and slaughterhouse microbiota: A comprehensive examination of resident microbial communities

Poster #	Poster Session 1: Microbiome diversity and food quality	Title
PS1-S6- PP01	Wisnu Adi Wicaksono	Exploring the diversity of bacterial communities in tempeh and its implications for food safety in Indonesia
PS1-S6- PP02	Irene Franciosa	Exploring microbial changes during green coffee fermentation in apple juice and their influence on coffee aroma
PS1-S6- PP03	Rain Kuldjärv	Is it possible to replace the traditional dynamic wild fermentation with preselected cultures?
PS1-S6- PP04	Moritz Hartmann	Application of transcriptionally active in-house microbiota on Austrian smear cheese: an early look
PS1-S6- PP05	Tiziana Nardi	Changes in grape-associated microbiome as a consequence of chitosan pre-harvest application
PS1-S6- PP06	Atefeh Asadi	Characterization of microbial diversity in ready-to-eat salad based on MALDI-TOF MS analysis and full- length 16S rRNA gene sequencing during cold storage
PS1-S6- PP07	Haktan Aktaş	An investigation on probiotic properties and safety evaluation of non-starter lactic acid bacteria from traditional Turkish yoghurt
PS1-S6- PP08	Benjamín Vazquez-Conejo	Delving into the potential of new <i>Lachancea thermotolerans</i> strains to improve the organoleptic profile of Pale Ale beer
PS1-S6- PP09	Ananya Gupta	Harnessing the microbiome for innovations in cocoa
PS1-S6- SF01	Francesca Cristetti	Exploration of microbial ecology as a quality marker through its linkage to the geographical origin of spontaneously fermented food matrices: The case of wine, green coffee beans, and cocoa beans
PS1-S6- SF02	Franz-Ferdinand Roch	Dynamic interactions between bacterial and fungal communities on vacuum packaged beef: A comprehensive longitudinal microbiome analysis
PS1-S6- SF03	Aashish Jha	Substrate drives densely connected microbial community assembly across diverse traditional fermented foods

Poster #	Poster Session 2: The edible microbiome	Title
PS2-S7- PP01	Madeleine Spatz	Ferments of the Future: A public-private partnership to accelerate research and innovation on ferments, fermented foods and biopreservation
PS2-S7- SF01	Matevž Zlatnar	Unravelling the fermented olives microbiome: Bacterial diversity, function and its importance for human health
Poster #	Poster Session 2: Microbiomes for improving health and well-being	Title
PS2-S8- PP01	Juan Lombardo-Hernández	Probiotic bacteria <i>Lactiplantibacillus plantarum</i> is detected by primary neural cortical cells inducing transcriptional, morphological and functional changes
PS2-S8- PP02	Hacer Meral-Aktaş	Determination of some <i>in vitro</i> probiotic properties of bacteriocinogenic <i>Enterococcus faecium</i> H108 and H206 strains
PS2-S8- PP03	Marwane Bourqqia Ramzi	Probiotics and gut microbiota-brain axis: Exploring bioelectrical communication through enterococci
PS2-S8- PP04	Jesus Mansilla	E. coli and the microbiota-gut-brain axis: Bioelectrical connection
PS2-S8- PP05	Robin Duncan	<i>In vitro</i> assessment of the effect of commercial botanical products on the human faecal bacterial community structure
PS2-S8- PP06	Elisa Di Stefano	<i>In situ</i> phytate degradation by <i>Bifidobacterium</i> spp. as a novel approach to tackle micronutrients deficiencies in early life.
PS2-S8- SF01	Gianfranco Picone	The application of High-Resolution Nuclear Magnetic Resonance (HR NMR) in metabolomic analyses of meconium and stool in newborns - the MABEL project: A preliminary study
PS2-S8- SF02	Nicola Mangieri	Role of fermented oat drink and fermented milk compared to freeze-dried cells in the survival of the probiotic strain <i>Lacticaseibacillus rhamnosus</i> CRL 1505 in human gastrointestinal transit
PS2-S8- SF03	Agapi Doulgeraki	Impact of an 8 weeks intervention with orange juice enriched with probiotics and vitamin D on the diet of volunteers at high cardiometabolic risk
Poster #	Poster Session 2: Microbiomes for plant fermentation	Title
PS2-S9- PP01	Eugenio Parene	The bacterial microbiota of table olives: A metastudy
PS2-S9- PP02	Despina Langari	Lactic acid bacteria ecology changes along the Spanish style cv. Chalkidiki green table olives spontaneously fermented under high and low NaCl conditions using classical microbiological and molecular techniques

PS2-S9- PP03	Elisa Di Stefano	Food matrix and disease prevention: <i>L. plantarum</i> fermentation as a tool to modulate bioactivity, digestibility and acceptability of pulse seeds
PS2-S9- PP04	Ana Miklavčič Višnjevec	New fermented food product with enhanced bioactivity to minimize green pea (<i>Pisum sativum</i> L.) byproducts
PS2-S9- SF01	Romane Junker	Understanding taxonomic diversity and functional signatures of fermented vegetables microbiome
PS2-S9- SF02	Afolake Olanbiwoninu	Metagenomic analyses revealed bacterial communities and diversities in fermented African locust beans
Poster #	Poster Session 2: Circular food systems for microbiomes improving animal, human and environmental health	Title
PS2-S10- PP01	Stephanie Schnorr	Searching for novel polyunsaturated fatty acid producers in terrestrial ecosystems implicated in rare-lipid provisioning and ecological services
PS2-S10- PP02	Elisa Bigarella	Effect of probiotic bacterial supplementation on growth performance and bioconversion ability of Hermetia illucens
PS2-S10- PP03	Antonia Bruno	Microorganisms for food tracking: The SeaTraceOmics project
PS2-S10- PP04	Marilena Budroni	Dynamics of the microbiota of brewers' spent grain during inoculated and spontaneous solid-state fermentation.
PS2-S10- PP05	Angela Sessitsch	MICROBIOMES4SOY: Healthier diets and sustainable food/feed systems through employing microbiomes for soya production and further use
PS2-S10- SF01	Soumya Sahai Saxena	Utilizing indigenous agricultural soil microbiota for the management of <i>Listeria monocytogenes</i> in Indian arable land
PS2-S10- SF02	Nelson Mota de Carvalho	Supplementation of agro-industrial by-products in animal feed towards well-fare and sustainability: Case study of sugarcane bagasse lignin
Poster #	Poster Session 2: Preservation of food systems microbiomes	Title
PS2-S11- PP01	Roberta Coronas	Catabolic footprint of the microbiome of Sardinian sourdough by OmniLog® technology
PS2-S11- PP02	Marco Garello	Validation of standard operating procedures for DNA extraction and microbiome analyses of soil samples
PS2-S11- PP03	Olga Nikoloudaki	Metabolic framework of spontaneous and synthetic sourdough metacommunities to reveal microbial players responsible for resilience and performance

PS2-S11- PP04	Massimo Ferrara	Cryopreservation of microbial consortia isolated from Apulian table olives: Effects on vitality and functional potential
PS2-S11- PP05	Sahar Maghrebi	Fermented sausage microbiome: Investigation, storage and exploitation
Poster #	Poster Session 2: Connectivity of microbiomes in the food system	Title
PS2-S12- PP01	Nicolò Cinti	Zonation of the Vitis vinifera microbiome in Vino Nobile di Montepulciano PDO production area



Opening Keynote

OP-PL01 Understanding soil microbiome response to climate change

Franciska De Vries

University of Amsterdam, Netherlands

Increasing extreme climatic events, such as droughts and heavy rainfall, threaten the functioning of food systems. Because soil microbes are key actors of soil biogeochemical processes, understanding their response to climate extremes is crucial for predicting the consequences for ecosystem



functioning, including their capacity to store carbon and provide nutrients for plants. Grasslands cover many parts of the world and are a crucial component of food production systems. In this talk, I will first go into the fundamental predictors of soil microbiome response to drought, freezing, flooding and heatwave – extreme events that are all increasing in frequency and severity with climate change. In a European-wide sampling of grassland soils and subsequent controlled exposure to simulated drought, freezing, flooding and heatwave, we found that these extreme events shifted soil microbiomes in distinct but consistent directions. The magnitude of these shifts could be predicted by soil and climatic conditions from the origin of these communities, where soil function was most strongly affected in regions that do not normally experience similar extreme conditions. These findings highlight that for understanding impacts of climate change on soil microbiomes and their functioning, we need to take into account local conditions. In the second part of my talk, I will go into the first results from a network of field experiments in the Netherlands in which we assess the impacts of drought and flooding on microbial communities and their functioning across grasslands on different soil types. After one season we found that summer drought turned soils briefly into a carbon source, while flooding and drought both affected forage quality, but in opposing directions. We are further investigating how different grassland affect soil microbial response to drought with the aim of incorporating beneficial species into grasslands for improving soil microbial functioning in a changing climate.



Microbiomes and the fertilizer crisis

S1-PL01 Enrichment for and selection of novel bacterial isolates with potential as biofertilizers for soil-less agriculture

Trevor Charles¹, Patricia Dörr de Quadros², Ally Miners¹, David Felipe Tello Yepes¹, Gabriele Bellotti³, Julie Hernández-Salmerón², Solmaz Komijani², Nicole Soja², Justin Carpani¹, Aranksha Thakor¹, Michael Lynch⁴, Jiujun Cheng⁴, Tanya Irani⁵

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- ² Earth Microbial
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⁵ Healthy Hydroponics InnoTech



Microbials hold tremendous potential for use as biofertilizers that can reduce the application of chemical fertilizers that are the backbone of modern high-yield agriculture. Increasingly, vegetable crop production is carried out under controlled environments, often without soil. For example, in Canada, about half of vegetable production is in greenhouses. Various types of hydroponic systems dose the crops with nutrients, and in many cases the fertilizer nutrient solution is recirculated, often with treatment aimed at reducing pathogens. This can disrupt the development of a beneficial microbial community. Microbial inoculants are sometimes added, but these are invariably strains that have been developed for field crops, and not well suited to hydroponics. In an effort to improve the microbiome of soil-less systems, aiming to reduce fertilizer requirements, reduce pathogens, and boost crop yield and quality, we have first characterized the microbiome of several commercial hydroponic systems. Guided by this information, we have then set out to isolate novel strains using enrichment cultures with the ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC) as sole nitrogen source, and seeded with soil or hydroponic nutrient solution. The ACC deaminase trait that facilitates ACC utilization is associated with plant growth promotion and stress reduction. We furthermore examined the effect of carbon source on the nature of the microbial community in the enrichment cultures, and isolates from those enrichment cultures are routinely subjected to genomic and phenotypic characterization. In a parallel effort, we have developed an enrichment method for Stutzerimonas isolates, many of which are diazotrophs, and some of which have plant growth promotion traits. Finally, through testing of mixtures of strains on different crops we look for synergic effects that can be used in the development of synthetic microbial communities (SynComms). Through our work, which combines microbial community analysis with inoculant supplementation, we aim to develop and maintain robust and healthy microbial communities in hydroponic systems that boost crop production even while reducing the requirement for chemical fertilizers. This would substantially increase the sustainability and profitability of vegetable production.

S1-ST01 *Lactuca sativa* genetic variation shapes the plant phenotypic and metabolic response to a soil microbial inoculum

Arianna Capparotto¹, Paolo Salvucci¹, Cristina Sudiro², Adriano Altissimo², Pieter Clauw³, Francesco Vuolo⁴, **Marco Giovannetti**⁵

- ¹ University of Padova, Italy
- ² Landlab Srl, Italy
- ³ Gregor Mendel Institute, Austria
- ⁴ Sacco Systems, Italy
- ⁵ University of Turin, Italy



Phosphorus (P) is an essential mineral for plants and one of the most important plant growth-limiting nutrients. Despite its large abundance in the soil, less than 20% is present in the inorganic form, easily available for plants. To face this limitation, phosphate-based fertilizers have been intensively used in the agricultural field since the beginning of the first green revolution. However, their main P source derives from phosphate rock, which is a limited and non-renewable resource. In the last few years, microbial-based approaches have been proposed as a possible solution to increase sustainability in food production technologies. Among these, the most promising candidates are arbuscular mycorrhizal fungi, with their ability to dramatically extend the root surface involved in the phosphate absorption, and phosphate solubilizing bacteria.

To unravel the plant genetic base of beneficial interaction with soil microorganisms, we monitored the impact of a microbial inoculum, consisting of two arbuscular mycorrhizal fungi and two phosphatesolubilizing bacteria, on a diverse panel of 128 fully sequenced *Lactuca sativa* plants under controlled conditions of phosphorus starvation. Emphasizing the pivotal role of plant genetic diversity in shaping responsiveness to microbial inoculants, our study integrated a range of physiological, metabolic, and biomass parameters to elucidate the plant's response and tracing back to the plant genome.

Among the varieties studied, approximately 10% exhibited statistically significant effects on plant growth and/or phosphate concentration, underscoring the intricate interplay between genotype and environmental factors. Particularly noteworthy is the variability observed across the entire panel, underscoring the importance of genotype-environment interactions.

Leveraging the wealth of phenotypic data collected, we conducted genome-wide association studies (GWAS), revealing genetic loci associated with plant responsiveness to the microbial inoculum and paving the way for selecting lettuce plants with a diminished need for phosphate and an improved interaction with beneficial microbes.

S1-SF01 Harnessing biostimulants and data-driven microbiome analysis to address the fertilizer crisis: A sustainable approach

Marta Acin-Albiac, Diego Rodriguez-de-Prado, Irene Adamo, Clara Fernández-Trujillo, Sam Röttjers, Patricia Jiménez Herrera, Beatriz García-Jiménez, Alberto Acedo Biome Makers, Spain

The escalating fertilizer crisis presents a critical challenge to global agricultural sustainability. The need to integrate eco-friendly products has risen among agroindustry stakeholders. Those products should enhance soil health, heavily influenced by the soil microbial community state, to ensure long-term food production with a rising demand. In this context, biostimulants represent a sustainable alternative by promoting beneficial microbial activity, enhancing plant nutrient uptake efficiency, while improving plant resilience. Hence, the development of products must be done from a microbiome perspective. In our study, we have developed an automated pipeline to precisely characterize products' impact on soil microbiome for agricultural experimental designs, using the most suitable statistical, robust and flexible methodology. In addition, we provide the results of product effects using BeCrop[®] microbiome indexes, which summarize key functions and aspects of microbiome in relation to plant health and crop production. The results could be easily and flexibly filtered going from a global point of view in several locations and crops, to a highly detailed view in each specific design factor combination. As a showcase, we characterized the effect in soil microbiome of a pair of biostimulants products in two different crops (soybean and Corn) across several locations. Our results demonstrate the potential of biostimulants to enhance crop resilience and productivity from a microbiome wide perspective, offering viable alternatives to traditional fertilizers. Our solution provides valuable insights for optimization of future agricultural interventions and can be smoothly communicated through BeCrop® indexes to different agroindustry stakeholders, such as product developers and farmers, to face environmental challenges.

This work is part of the project PTQ2022-012402, funded by MCIN/AEEI/10.13039/5001100011033 and by the European Union "NextGenerationEU"/PRTR.

S1-SF02 Potential of microbiome-based solution as green biofertilizer for sustainable maize productivity

Annamaria Bevivino¹, Jonas Hett², Mariolina Gulli³, Lisa Cangioli⁴, Alessia Fiore¹, Nils Kleinbölting⁵, Manuela Costanzo¹, Alex Sczyrba⁵, Andreas Schlüter⁵, Silvia Tabacchioni¹, Andrea Visca¹, Elena Maestri³, Daniel Neuhoff², Nelson Marmiroli³

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- ⁴ University of Florence, Italy
- ⁵ Bielefeld University, Germany

Increasing crop production without the use of environmentally harmful products as chemical fertilizers is a major challenge of the 21st century. The employment of microbial consortia (MC) made of plant growth-promoting microorganisms offers an environmentally friendly alternative to the use of inorganic fertilizers. The main objectives of the present work are to exploit the potential of specifically selected MC with multifunctional properties for attaining sustainable agricultural production systems, to assess the impact of their application in field on indigenous rhizosphere microbial diversity and to evaluate whether natural soil microbiomes are negatively or positively affected by adding foreign microorganisms. MC were applied alone or in combination with Arbuscular Mycorrhizal Fungi and Biochar in two-years field trials in open field under conventional and organic management, and compared with commercial microbial products. The plant growth, diversity and composition of the maize rhizosphere microbiome and relative abundances of taxa were investigated at different maize growth stages and with different fertilization levels. The application of MC exerted a positive effect on plant growth especially at lower fertilization levels, while it did not significantly affect species diversity and richness of the native rhizosphere microbial communities. A great impact of biochar on rhizosphere soil microbiome was found suggesting that functionalization of biochar with MC seems a promising approach for microbiome modulation and enhancing plant growth. WGS metagenome sequencing revealed slightly significant differences in community composition only between low and high fertilization levels and a significantly higher relative abundance of reads assigned to the SEED category "Nitrogen fixation" at low fertilization level. Finally, genome sequencing of strains composing MC excluded any potential risk associated with scaling-up and their commercial application. Overall, our results suggest that multifunctional MC may be effectively exploited as green biofertilizer in sustainable maize cultivation without altering the biodiversity of the resident microbiota, thus avoiding risks of long-term impacts on natural biodiversity.

Acknowledgments

This work has received funding the Italian project SOIL-HUB (funded by MIPAAFT, D.M. 35851 del 5/11/2019), and from the the EU Horizon 2020 research and innovation programme under GA No. 818431 (SIMBA) and No. 652615 (EJP SOIL).



S1-PL02 What can microbial plant biostimulants do for our food quality: The mycorrhiza-tomato story

Carolin Schneider¹, Imke Hutter¹, Bettina Hause²

¹ Inoq GmbH, Germany ² Leibniz Institute of Plant Biochemistry, Germany

Industrialized tomato production faces a decrease in flavours and nutritional value due to conventional breeding. Moreover, tomato production heavily relies on nitrogen and phosphate fertilization.



Phosphate uptake and improvement of fruit quality by arbuscular mycorrhizal (AM)fungi are wellstudied. We addressed the question of whether commercially used tomato cultivars grown in a hydroponic system can be mycorrhizal, leading to improved fruit quality. Tomato plants inoculated with *Rhizophagus irregularis* were grown under different phosphate concentrations and in substrates used in industrial tomato production. Changes in fruit gene expression and metabolite levels were checked by RNAseq and metabolite determination, respectively. The tests revealed that reduction of phosphate to 80% and use of mixed substrate allow AM establishment without affecting yield. By comparing green fruits from non-mycorrhizal and mycorrhizal plants, differentially expressed genes (DEGs) were found to possibly be involved in processes regulating fruit maturation and nutrition. Red fruits from mycorrhizal plants showed a trend of higher BRIX values and increased levels of carotenoids in comparison to those from non-mycorrhizal plants. Free amino acids exhibited up to four times higher levels in red fruits due to AM, showing the potential of mycorrhization to increase the nutritional value of tomatoes in industrialized production.

S1-ST02 The microbiome associated to the weed *Panicum* sp. exploited as self-produced sustainable biofertilizer for staple crops in Sri Lanka

Lorenzo Vergani², Mekhala Chandrasekara¹, Chathurika Umayangani¹, Francesca Mapelli², Sanath Hettiarachi¹, **Sara Borin²** ¹ Rajarata University, Sri Lanka ² University of Milan, Italy

Climate change and interlinked soil degradation slowed global



agricultural productivity, reducing crop yields and threatening food security. In this context, smallholder farmers in the Global South are disproportionally affected and urge the need for sustainable agricultural practices to reduce the use of toxic chemical fertilizers, while maintaining the ecosystem services given by healthy soils and biodiversity, together with the improvement of crop resilience to environmental threads. "Smallholder-friendly" biofertilizers are a promising approach to counteract the high fertilizer costs and maintaining human and ecosystem health. Robust and hypothesis-based experimental research coupled with in field feasibility studies are nevertheless needed. This work, in the frame of an international cooperation project, aimed at covering this gap and focused on the evaluation of a low-tech microbiome-based fertilizer, self-produced by farmers in Sri Lanka. The rational of this biofertilizer is that invasive weeds should harbour a microbiome conferring resilience and stress tolerance to the host, which could be exploited as a biofertilizer for crops. Root wash (RW) and arbuscular mycorrhizae (AMF) inocula from Panicum sp. were hence prepared and supplied by Sri Lanka farmers to different crops such as chili, and local rice varieties, with decreasing doses of chemical fertilizer in several field trials. The treatments improved the crop yield compared to the non-treated controls or to the plants cultivated with the minimum dose of chemical fertilizer. Both RW and AMF treatments supplied with low fertilizer dose increased plant productivity at the same level than complete chemical fertilization. The rhizosphere microbiota of crops was analysed at different growth stages by Illumina amplicon sequencing applied to the bacterial and fungal fractions. The biofertilizer treatments modulated the rhizosphere microbial communities, which showed a different structure from the non-treated plants. Taxa present in RW and differentially enriched in the treated plant rhizosphere were identified, demonstrating that the Panicum microbiome was able at some extent to colonize crops. Overall, this experimentation allowed to test in real conditions the feasibility of the approach, together with raising awareness on the microbiome potential in sustainable agriculture intensification.



Microbiomes mitigating biotic and abiotic stress

S2-PL01 What the olive tree holobiont needs to confront a soilborne fungal pathogen (and how to help it)

Jesús Mercado-Blanco

CSIC, Spain

Olive (*Olea europaea* L. subsp. *europaea*) is one of the most significant tree crops in temperate areas worldwide, constituting an agro-ecosystem of major relevance in the Mediterranean Basin. Olive oil and table olives are a significant part of the so-called Mediterranean diet. A number of



benefits for the human's health are linked to the consumption of extra virgin olive oil (e.g. lower rate of some types of cancer and cardiovascular disease) and table olives (e.g. source of probiotics). Many traditional and emerging (a)biotic stresses such as changing climatic conditions, drought and/or the presence of pathogens and pests pose a threat to this crop. Among them, Verticillium wilt of olive (VWO), caused by the soil-borne fungus Verticillium dahliae Kleb. is considered one of the main limiting factors for olive cultivation. This disease is very difficult to control due to a multiplicity of factors. Therefore, the implementation of an integrated disease management strategy is strongly recommended, with emphasis in preventive actions. Within this framework, the use of tolerant olive cultivars constitutes one of the most promising, environmentally friendly and economically profitable control approaches. In this talk, the relationship between the tolerance/susceptibility to VWO and the olive root system as a whole will be summarized. That is, considering morphological, biochemical, genetic and root-microbiome perspectives. It will be concluded that the tolerance of a given olive cultivar depends on the sum of structural, physiological and biochemical constitutive traits that block, or at least slow down, the V. dahliae invasion process. Furthermore, the effectiveness and speed of the olive tree to further activate specific defence mechanisms at the belowground level (e.g., gene expression, lignin deposition, quantitative changes in secondary metabolites content, cell membrane permeability adjustment) upon V. dahliae attack is also decisive. The composition of the olive root microbiome and, mainly, co-occurrence interaction changes occurring among its members in response to external perturbations (i.e. pathogen and/or inoculation with biocontrol agents), also decisively influence the performance of the olive holobiont to confront V. dahliae. These findings can contribute to generate innovative and more holistic alternatives for the effective management of VWO.

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S2-PL02 The role of phenotyping facilities to analyze plantmicrobial interactions and their access possibilities across Europe

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Plant-microbe interaction depends on a multitude of parameters and has



to be studied across scales from simplified (highly controlled) systems up to complex field conditions to dissect the mutual interaction, investigate the mechanisms and translate the understanding into application. This requires quantitative measurements of the plant phenotype and the biotic and abiotic environment in high throughput.

At IBG-2 we develop, apply, and provide access to facilities using non-invasive technologies for the characterization of plant performance under highly controlled environmental conditions and field applications. This includes plant-microbial cultivation systems of different complexity such as agar, paper pouches, soil-filled tubes in greenhouses or soil in fields, precise environmental monitoring, and information technology for data management. The use of non-invasive phenotyping facilities allows the detection of above- and below-ground plant traits on an individual plant and plot level.

Here, we include phenotyping examples to show that phenotyping facilities enable innovative approaches to advance mechanistic and functional understanding of plant-microbial interactions. We address questions like nutrient uptake, temperature changes, effects on growth as well as characteristics of root and shoot architectures in 2D and 3D. We show an overview of research infrastructure projects and initiatives, which provide users with access to phenotyping facilities across Europe to facilitate cross-disciplinary research within agroecology, microbiome, and biodiversity implications.

S2-ST01 The role of microbiome shift on the emergence of climate resilient coral species

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Climate change-induced rise in sea surface temperatures has led to an increase in the frequency and severity of coral bleaching events, ultimately leading to the deterioration of coral reefs, globally. However, the reef-building corals have an inherent capacity to acclimatize to



thermal stress on pre-exposure to high temperatures by "switching" or "shuffling" their symbiotic dinoflagellate - Symbiodiniaceae community towards a thermal tolerant composition. This reorganisation may become an important tool in coral's resilience to rapid environmental change. Therefore, it is crucial to delineate the Symbiodiniaceae community in our reef to predict their resilience capacity. Our study aims to analyse the symbiont community associated with common reef corals in a heat-stressed, marginal turbid reef of Palk Bay, India. We employed next-generation sequencing-based high-resolution analyses of internal transcribed spacer two (ITS2) amplicons within the SymPortal framework to examine the diversity and organization of Symbiodiniaceae communities. The results revealed a dominance of heat-tolerant Durusdinium (D1-D4) and Cladocopium (C15) across coral species (Acropora cytherea, Acropora digitifera, Favites abdita, and Porites lobata) and reef environment (seawater, and reef sediment), with the presence of 18 ITS2 type profiles in our sample. To our knowledge, this is the first comprehensive study to delineate Symbiodiniaceae and associated microbial communities at fine scale resolution (ITS2 type profile) from scleractinian corals of India and provides a baseline for future work in a marginal reef ecosystem of India. Several studies have highlighted the importance turbid reefs as prospective climate refugia. Therefore, this study is crucial for researchers, scientists, stakeholders, and policymakers to identify reef sites and coral species with resilience capacity for devising future reef restoration and management strategies.

S2-ST02 Plants 'cry-for-help' in presence of the xenobiotic polychlorinated biphenyls

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Together with land overuse and climate change, pollution represents one of the main causes of biodiversity loss and soil degradation. Polychlorinated biphenyls (PCBs), like many other anthropogenic pollutants, are xenobiotics with carcinogenic and recalcitrant properties



and represent a harm for ecosystem health. Their removal can be achieved through rhizoremediation, a process resulting from the beneficial interaction between plants and degrading microorganisms and that is putatively mediated through a 'cry-for-help', consisting in the remodeling of the root exudate (RE) chemistry to recruit bacteria able to alleviate PCB-induced phytotoxicity.

We investigated REs influence on the services provided by degrading bacteria to the plant in presence of PCBs. It was observed that REs like flavonoids, supplied as pure chemicals, could promote in vitro the recruitment and persistence in the root system of the PCB degrader P. xenovorans LB400, by improving its proliferation, swimming motility, chemotaxis and biofilm formation. Indeed, early root colonization was enhanced in the flavonoid over-accumulating Arabidopsis mutant line tt8. Moreover, 100 µM flavone and quercetin activated the transcription of the PCB catabolic bphA gene, further supporting the degrading functionality of strain LB400. The bacterium efficiently colonized Arabidopsis roots and showed plant growth promoting effects under control conditions and in presence of 20µM PCB-18. Nevertheless, these beneficial effects were not affected in Arabidopsis mutant lines with altered flavonoid exudation profiles, leading to the conclusion that non-flavonoid REs might be involved in later stages of plant-microbe interactions under PCB stress. A metabolomic approach was performed to identify the PCB-driven signature in root chemistry. Among the identified metabolites, the exudation of scopoletin, an antimicrobial coumarin inhibiting some of the known PCB degrading bacteria, decreased in presence of PCB. Instead, over-exuded metabolites were instead used as C and N sources by bacteria. These results suggest that the plant 'cry-for-help' under PCB stress may reshape the root chemistry to provide more suitable growth conditions for degrading strains. By increasing the knowledge about the holobiont dynamics in polluted soils, tailored rhizoremediation procedures may be implemented, by engineering the degrading microbiome and through the selection of plant species with specific exudation patterns.

S2-ST03 Unveiling the microbial players: Metagenomic insights into olive varieties' response to drought

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The worldwide rise in water scarcity poses a significant challenge to maintaining crop productivity. Insufficient water leads to the deterioration of the photosynthetic machinery, disruptions in crucial metabolic pathways, heightened production of free radicals, and a weakening of plant root architecture. Drought stands out as a primary stressor directly impacting the osmotic balance of plant cells. In the context of the BIOMEnext Project, the implementation of innovative, composite and ecofriendly farming systems, in order to enhance the resilience of Mediterranean fruit farming to climate change, has been evaluated. In particular, metagenomic analyses, characterization of the core rhizosphere and endophytes microbiomes and their predicted functions on four olive variety (Arbequina, Chemlal, Koroneiki and Shengeh) growth on wet and dry condition in a one-year field experiment was performed. The DNA from both root endophytes and soil rhizosphere was extracted and sequenced using the Oxford Nanopore technology, producing long reads of the gene 16S rRNA. The long reads made it possible to identify the microbial composition at specie level using the Emu tool. Moreover, a novel tool has been developed to perform the functional annotation using PICRUSt2 with long reads. Interestingly, up to 2752 different microbial species were found in rhizosphere and root endophyte samples. The most abundant ones were Vicinamibacter silvestris, Microlunatus phosphovorus and Microvirga tunisiensis, which are known to be typical root nodule symbiotic bacteria of subtropical savannah soils, able to accumulate polyphosphate. These findings lay the groundwork for addressing the issue of drought in arid and semi-arid regions, utilizing natural solutions such as selected microbial consortia enriched with indigenous strains capable of tolerating extreme drought conditions and still providing benefits to plants.

Acknowledgments

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S2-SF01 Isolation and selection of plant-associated microbes for the formulation of new inocula to be used in sustainable agriculture

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The current climate change is putting agricultural lands at higher risk of suffering from severe abiotic stress, among which drought plays a major role. Furthermore, the continuous use of chemical fertilizers in cultivated fields has caused a decrease in soil microbial diversity. An emerging solution consists in the application of plant microbial biostimulants: such products are composed by a combination of diverse matrices (often organic substances based on by-products) and microorganisms, such as rhizobacteria and/or arbuscular mycorrhizal fungi (AMF).

The main objective of this project is to isolate and select from agricultural soils active components of the root microbiota, with a special focus on drought stress conditions, for potential agricultural applications. Two different crops were employed *Solanum lycopersicum* and *Oryza sativa*. Plants were grown in a substrate made of a pool of soils coming from harsh environments. Drought stress was gradually induced and then a recovery phase was applied. Next-generation sequencing techniques, performed on rhizosphere and endosphere samples, allowed the microbial biodiversity (bacteria and fungi) description during drought stress and after the recovery phase. The results obtained suggest a deep shaping of the microbial community upon drought, and indicate that the recovery phase can efficiently restore a higher level biodiversity under the tested experimental conditions. In particular, the Bacillus genus turned out to be more abundant under drought stress.

In parallel, a culture-dependent approach has been followed. Sporogenic bacteria were isolated from rhizosphere and endosphere of both crops. The ones belonging to Bacillus genus were selected to perform different tests (P solubilization, IAA production, siderophores production, drought tolerance potential, ACC deaminase potential) in order to assess their plant-growth promoting abilities. As a second step, the AMF component present in the rhizosphere samples is isolated following the "trap culture" method, and characterized. The results obtained highlight the potential PGP abilities of the isolated strains, and will contribute to find new solutions, aimed at counteracting the rising climate changes, in a perspective of sustainable agriculture.

S2-SF02 Effect of different beneficial microorganisms in the growth promotion of camelina (*Camelina sativa* L. Crantz)

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In recent years, abiotic stresses such as prolonged drought periods and high temperatures have resulted in a decline in crop yields in the Mediterranean region. In Europe, there is an urgent need for differentiating oilseed production in order to address the reliance on imported raw materials, for both feed and food industry. Camelina could be a viable choice, due to its low input agronomic management in terms of nutrients and strong resistance to drought. The seeds have a high oil content (35-45%), and the oil composition is unique and allows different food and non-food applications to achieve the goals set by the new European Green Deal, which aims to reduce fertilizer use by 2030 and minimize nutrient losses in the soil, one potential solution could be the implementation of PGPR (Plant Growth Promotor Rhizobacteria). This approach would enhance crop yields without the need for additional agricultural inputs. This study was conducted to observe the effects of two commercial camelina varieties (Alba, supplied by Camelina Company, Spain, and Cypress, supplied by Smart Earth Corp, Canada) and different PGPR: P. phytofirmans (11), A. brasilense (12) and B. toyonensis (13) in a controlled environment experiment. The study aimed to assess the interactions between the camelina varieties and PGPR, as well as their response to abiotic stress caused by two different water regimes (wellwatered and drought) applied after flowering. Seed production was affected by the PGPR application, the stress imposition and the interaction "var"x"stress" and "stress"x"PGPR". Concerning "stress"x"PGPR", seed production decreased under well-watered conditions when I2 and I3 were inoculated compared with the non-inoculated control. Under drought, I3 had the highest seed production $(0.50 \text{ g plant}^{-1})$ compared with the non-inoculated plants and the other treatments (0.3,0.3, and 0.4 g plant⁻¹ for control I1 and I2, respectively). Oil content was affected by the interaction "var"x"PGPR": Alba exhibited greater stability across all treatments, with an average oil content of 31%. On the other hand, Cypress showed higher oil contents of 33% when treated with I3 compared with the control (31%). The results indicated that the combination of PGPR and variety, as well as PGPR and water regime, had a significant impact on both quantitative and qualitative parameters. Therefore, the application of PGPR in camelina, especially under abiotic stress, could improve yield parameters.



Animal welfare, antibiotic resistance, and robustness

S3-PL01 Age matters: Exploring differential effects of antimicrobial treatment on gut microbiota of adult and juvenile brown trouts and non-target organisms of the aquatic ecosystem

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Amid the growing demand for sustainable protein-rich nutrition, particularly in regions disproportionally affected by the ongoing food crisis, aquaculture has become a pivotal resource. Antibiotics and Antiparasitics, extensively used in hatcheries to maximize production yields and as surrogates for inadequate hygiene, have been linked to increased abundance of antibiotic resistance genes and persistent shifts in the intestinal microbiome of various farmed fish species. Given that the gut microbiome of juvenile fish is still maturing, it likely exhibits greater susceptibility to external perturbations compared to the more stable microbiome of adult fish. Using a metabarcoding approach, this study focuses on the distinct impact of antimicrobial treatment on the gut microbiome of juvenile and adult brown trouts (Salmo trutta fario), exploring the co-selective pressure of combined florfenicol (FF) and peracetic acid (PAA) application as well as the influence of age. In a freshwater RAS, one and two summer-old brown trouts underwent three treatments (10 mg/kg FF via medicated feed, FF+PAA, PAA) compared to an untreated control group. Faecal samples were collected prior to administration, on the last day of administration (day 10) and four times during the post-treatment phase, followed by 16S rRNA gene based amplicon sequencing on the Illumina MiSeq platform. Results show distinct changes in the gut microbiome composition of juvenile trout following both FF and PAA treatment, marked by decreased abundance of several beneficial core taxa (Shewanella, Lactococcus, Deefgea) and an increase of putative pathogens (Streptococcus, Acinetobacter). Notably, these effects were absent in adult fish. Addressing a critical knowledge gap regarding the extent to which the response to antimicrobial treatment is dependent on the developmental stage of the fish, this study emphasizes the importance of understanding differential effects between developmental stages and highlights the potential long-term consequences of combined application of antibiotics and antiparasitics in aquaculture.
S3-PL02 Microbiomes: Friend or foe in the battle against antimicrobial resistance

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Antimicrobial resistance (AMR) poses a major threat to animal and human health, causing 1.27 million human deaths in 2019, with over 10 million deaths predicted by 2050. The World Bank also predict that AMR bacterial infections will cause a reduction in global livestock production



of 3–8% by 2050, being more prominent in developing countries with an 11% reduction expected in these countries. AMR cannot be studied in ecosystem silos as an AMR bacterium can occupy multiple niches, and therefore requires a 'one health' approach to monitor and control threat.

Recently, we identified 235, 101, 167 and 182 different resistance genes in metagenomes obtained from poultry, ruminant and swine gastrointestinal tracts (GITs), as well as in soil respectively (5,800 metagenomes representing 37 countries), with 55 of the genes being found across all sample types¹. Tetracycline resistance genes were the most widespread in the livestock GIT microbiomes, which was confirmed within metatranscriptome sequences for the same sample types. Conversely, Oleandomycin resistance genes were most abundant in the soil metagenomes, which was also confirmed in metatranscriptomes from the same sample types. In an another study utilising antimicrobial testing and computational techniques on 435 rumen bacterial isolates and genomes we again found a high abundance of tetracycline resistance genes and evidence that the *tet(W)* gene is under positive selective pressure being located on a novel integrative and conjugative element in several ruminal bacterial genomes².

Although livestock microbiomes pose a potential threat to animal and human health, we have also shown that they are reservoirs of novel antimicrobials, rife for therapeutic development against AMR bacterial pathogens^{3,4,5}. For example, we identified >200 novel antimicrobial peptides from the rumen microbiome, with therapeutic activity against numerous pathogens, including *Staphylococcus aureus*, *Acinetobacter baumannii* and *Pseudomonas aeruginosa*^{3,4,5}. Therefore, microbiomes containing AMR bacteria are not just a threat for animal human disease and can also be part of the solution.

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S3-ST01 Longitudinal study of chicken microbiomes from egg to meat: Impact of farming practices.

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Chicken is the most consumed meat worldwide, with a variety of farming schemes from intensive indoor to free-range systems which raises different concerns in terms animal health and welfare. Recent studies



suggest that microbial fluxes in chicken are influenced by the aviary environment, the diet, the breeder's lineage and that various and successive microbial contamination or dispersion events occur in the chicken breeding life. A better understanding of these fluxes could help to identify potential levers for controlling the microbiome and guaranteeing the health and robustness of animals.

Our project used a large-scale longitudinal study from egg to meat to study the impact of two farming practices: conventional claustration and outdoor access. The microbiome of a hundred animals (males & females), half of them having outdoor access, were analysed from hatch until slaughter at 61 days. Four types of samples were analysed (aviary environments, *caeca*, droppings and carcasses) using 16S rDNA amplicon sequencing. A total of 600 microbiome samples were compared between both farming modalities.

Overall, the chicken bacterial diversity in the dataset was evaluated to 2256 species (207 genera). Our results on longitudinal droppings sub-dataset indicate that the gut microbiome of chicken undergoes two important maturation steps, independently of the farming practice, one taking place a few days after hatching and the second taking place around the fourth week of farming. These two steps were characterised by a strong increase in bacterial diversity, the second one being the most important.

Outdoor access also impacted the microbial composition in *caeca* and droppings, but mainly after 4 weeks of outdoor access. Compared to claustration, outdoor access increased significantly the microbial diversity of the chicken gut microbiome, whereas carcasses' microbiome did not show any significant difference. *Caeca* samples were those showing the strongest differences between the farming practices indicating that the difference in the diet (due to the farming practice) was the main factor influencing the chicken gut microbiome. Detailed analysis in these differences will be presented.

[#] Stéphane Chaillou, Cecile Berri, Thierry Bordeau, Romain Briandet, Estelle Cailleau-Audouin, Emilie Chambellon, Michel Duclos, Karine Germain, Emmanuelle Helloin, Alice Hondelatte, Laure Ravon, Olivier Rué, Pascale Serror, Monique Zagorec.

S3-ST02 The resistome investigation in foods and their processing environments from 113 European companies reveals high connection with mobile genetic elements and ESKAPEE bacteria

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Despite the importance of food as a source of transmission of antimicrobial resistance (AMR) determinants into humas, the resistome occurring in food producing environments is still poorly understood. Here, we sequenced and analysed the resistome from 1,780 samples of raw materials, final products and industrial surfaces (from different processing rooms) collected at 113 European facilities across five countries (Austria, Iceland, Ireland, Italy and Spain) producing cheese, meat, vegetables and fish products. This represents the most ambitious survey of the resistome of foods and associated processing environments up to now, considering the number of samples collected (that expanded ~3x public available food-related metagenomes), the sequencing approach and depth (~2x depth, which yielded ~4x number of MAGs recovered). We aimed to characterize the resistome in food and food production surfaces both quantitatively (assembly-free approach and calculation of CPM), in order to infer the foods, materials and/or surfaces that contribute the most to the resistome, and qualitatively (assembly-based approach and analysis of contigs and MAGs), in order to infer in the taxa associated with the resistome and the relationship of AMRG with MGE. More than 70% of the known (according to ResFinder database) AMR genes (AMRG), were detected (including those conferring resistance to the most commonly used antimicrobials in EU and those that are considered of critical importance by the WHO) and showed tetracyclines, beta-lactams, aminoglycosides and macrolides AMRGs as the most abundant overall. The evaluation of the different production systems showed meat industries to harbour greater abundance and diversity of AMRG than dairy, vegetable and fish industries. The taxonomic analysis revealed ESKAPEE bacteria, Staphylococcus equorum and Acinetobacter johnsonii as the main AMRG-carrying microbes overall. The evaluation of the mobilome of AMRG is a critical aspect, due to their relationship with increased transmission, and showed that 45% of the AMRG were carried on mobile genetic elements (MGE), mainly plasmids, transposons, insertion sequences and integrons. Our results also showed the impact of certain surfaces and rooms from the producing facility on shaping the resistome of the final products and highlight the strength of culture-independent microbiome analyses for the untargeted identification and characterisation of food safety hazards.

S3-SF01 Deciphering microbial dynamics and antimicrobial resistance spread in beef production

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The environmental and socio-economic implications of the food industry pose critical concerns regarding both food waste and antimicrobial resistance (AMR). Microbial-induced spoilage is a significant contributor to food loss, and the use of antimicrobials is known to be one of the main drivers of AMR spread. However, there is a notable lack of direct research on AMR spread within food systems. In this research, we used a deep shotgun metagenome sequencing approach to obtain a functional profile of the microbial community found on different surfaces (including conveyor belts, membrane skinners, etc.) in an Austrian beef processing facility during processing. The metagenomic analysis revealed a significant prevalence of antimicrobial resistance (AMR) genes, predominantly associated with Enterobacteria. These genes are associated with resistance to some of the most commonly prescribed and consumed antibiotics, including aminoglycosides (42.1% of the total AMR genes; mainly *aph(6)-Id* and *aph(3'')-Ib*), beta-lactams (31.6%; mainly different *blaOXA*), tetracyclines (10.5%) and amphenicols (7.0%). Taxonomic profiling of the microbiota from the different samples, targeting potential pathogens and/or spoilage microbes, revealed the presence of *Actinobacter* spp., *Psychrobacter* spp., *Pseudomonas fragi* and *Brochotrix thermosphacta* in the facility. Noteworthy, their abundance increased throughout the three time points collected within the same working shift.

The findings of this research offer better insight into the dynamics of contamination dissemination within beef processing facilities, providing valuable insights into AMR prevalence, the metabolic potential of facility microbiota and strategies to enhance hygiene standards and meat quality, thereby reducing food waste. Our samples were selected *ad hoc* after a thorough inspection of the meat processing facility to cover a wide variety of surfaces towards the identification of AMR and spoilage hotspots, detection of virulence factors and genes involved in biofilm synthesis. By different risk potentials, our study is elucidating the path of microbe dissemination, highlighting the underlying risks posed by microorganisms, regardless of their pathogenic attributes.

S3-SF02 Unravelling gut dynamics: Investigating the impact of a novel supplement on canine gut microbiota

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Oxidative stress has been implicated in the pathogenesis of many gastrointestinal (GI) tract disorders in humans and animals such as Dysbiosis, Acute and Chronic Diarrhea, Leaky gut syndrome and many more.

The GI tract harbours a diverse community of microorganisms, collectively known as the gut microbiome, influencing canine health. Perturbations in this microbial ecosystem are implicated in the development of several GI disorders. Treatment of GI tract disorders usually involves the use of antiinflammatory drugs and antibiotics. However, the use of antibiotics can induce antibiotic resistance. Studies in human and animal models have hypothesized the use of natural products with antioxidant properties as a possible alternative or support to common drugs used in the treatment of oxidative stress. This study aimed to investigate the effects of a newly formulated supplement on the composition of gut microbiota in healthy adult dogs. The supplement, known for its ability to enhance canine gut health, comprises antioxidant-rich natural ingredients including bromelain, quercetin, and *Lentinula edodes*.

In a randomized controlled trial, adult healthy dogs were allocated to either the supplement (TRT, n=15) or a placebo (CTR, n=15) over 28 days. Stool samples were collected at baseline (T0), Post-supplementation (T28), and one-week post-supplementation (T35) for 16S rRNA sequencing analysis. Alpha- and beta-diversity metrics were assessed using QIIME2, while taxonomic differences pre- and post-supplementation were evaluated through ANCOM-BC analysis.

A significant reduction in overall diversity was observed in the CTR group, contrasting with the stable diversity observed in the TRT group over time. Moreover, distinct shifts in taxonomic abundances were evident in both groups, with a more pronounced increase in beneficial bacterial taxa noted in the TRT group. Notably, genera such as *Bifidobacterium, Lactobacillus,* and *Pediococcus* exhibited notable augmentation at T28 in the TRT group, with sustained increases in *Bifidobacterium* and *Lactobacillus* at T35 compared to T0. These taxa are recognized for their anti-inflammatory properties and association with canine gut health.

In summary, our findings underscore the potential of the new supplemented formulation to selectively modulate specific beneficial bacterial taxa, offering a targeted approach for gut microbiome modulation without disrupting its overall equilibrium.



Microbiomes and (GHG, manure) emissions

S4-PL01 Unlocking microbiomes for reduced emissions in sustainable agriculture

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Microbiomes occur everywhere in natural and cultivated ecosystems, such as soils, plants, animals, and our bodies. Here they play essential roles and can contribute to sustainable solutions to societal challenges,



such as zero-hunger, reversing biodiversity loss and climate mitigation. In order to realize this potential, however, a transition to holistic systems approaches in microbiome research is urgently needed to fully leverage microbiome functions. This involves understanding how microbiome modulation affects the ecosystem studied as well as connected ecosystems. Furthermore, it requires understanding of how microbiome modulation results in desirable functions needed as a basis for the transition towards a sustainable and circular bioeconomy. To this end, we recently established the Wageningen Microbiome Center, that envisions to act as a new interdisciplinary interface for innovation within the (inter)national microbiome research community and to contribute to subsequent valorisation by societal and industrial partners. In this presentation, examples will be provided illustrating the role of microbiomes in the production and release of emissions in different domains of agri- and horticultural production systems, and how knowledge-based microbiome management can contribute to solutions for reducing emissions in sustainable production systems.

Furthermore, knowledge of functional roles of individual microbial populations and their interactions is crucial to achieve the desired improvement in our understanding of microbiomes and their applicability for managing ecosystem health and functioning. In order to enable breakthrough research and knowledge sharing on natural and defined (model) microbial communities, we established UNLOCK, a large-scale facility that integrates experimental and data platforms, ranging from single-cell based approaches to complex community studies. UNLOCK is open to excellence-driven users from universities, knowledge institutes and industries, placing them in the unique position to conduct research at unmet speed and resolution. Specific examples illustrating the UNLOCK concept will be given from dedicated studies of natural as well as synthetic minimal microbiomes.

S4-PL02 The role of the rumen microbiome in the development of methane mitigation strategies for ruminants

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The rumen microbial community provides ruminants with a unique ability to convert human indigestible plant matter, into high quality protein. However, methane (CH_4), a potent greenhouse gas, estimated to have a



100-year global warming potential 27 times greater than that of carbon dioxide (CO₂), is produced as a metabolic by-product of ruminal fermentation of feed by rumen microbes. As the rumen microbiome constitutes 15-40% of the inter-animal variation in enteric CH₄ emissions, understanding the fundamental microbiological mechanisms underpinning ruminal methanogenesis is crucial for developing CH₄ mitigation strategies. Both the composition and activity of the rumen microbiome has been shown to vary between high and low methane emitting animals. For example, an increased abundance of a symbiotic cohort of bacteria capable of producing lactic acid, and subsequently converting it into propionate, as well as an methanogen community associated with a low hydrogen environment, has been observed in low methane emitting beef cattle. Indeed, the contribution of the rumen microbiome to the methanogenic output of an animal is further exemplified by the fact that the relative abundance of three ruminal bacteria and the *Methanobrevibacter* SGMT clade, has been shown to be accountable for 20% of the variation in CH₄ emissions.

Host genetics and the rumen microbiome are intrinsically linked to ruminal fermentation and the methanogensis process. As a result, microbiome assisted genomic approaches are currently under investigation as a tool to further enhance the breeding values for enteric CH₄, which are currently being included as part of national breeding programmes. Equally, numerous feed additives, with anti-methanogenic properties, have been identified as effective CH₄ mitigation measures, however additional benefits to animal performance are yet to be identified. Furthermore, investigating the possibility of early life modification of the rumen microbiome and its potential as a long lasting CH₄ mitigation strategy for ruminants, is actively under investigation. By further advancing our understanding of the establishment, composition and functionality of the rumen microbiome, the potential exists to both improve the effectiveness of existing, and develop new innovative, CH₄ mitigation strategies for the ruminant livestock sector.



Food systems microbiomes and epidemics

S5-PL01 Food system and environmental microbiomes, a bedirectional connection.

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It is now a matter of the fact that the microbiomes from food systems and environments are intimately interconnected. The firsts certainly depending on the seconds, but, also, influencing their balance, establishing a feedback process with concrete implications for the



environmental health. Here we will explore some examples of these intimate trans-microbiomes bedirectional interactions, as well as their cascade impacts on food safety and quality. In particular, we will explore the routes of dispersion of antibiotic resistance genes from the farm systems to the surrounding environments up to the local human population, suggesting the association with the workers microbiomes as the main route of dispersion. Analogously, we will see how mussels (*Mytilus galloprovincialis*) farmed in polluted marine waters, select, enrich and disperse antibiotic resistant bacteria, representing a treat for the health of the surrounding marine ecosystem. Conversely, we will provide some examples on the importance of environmental microbiomes as a source of the natural microbial biodiversity to support the sustainable production of high-quality foods. More specifically, the importance of the local diversification of soil microbiomes in the Vino Nobile di Montepulciano PDO production area will be explored, as well as the relevance of microbiomes of the local marine sediments for the health and productivity of clamps (*Chamelea gallin*) in the North Western Adriatic Sea

S5-ST01 Exploring the occurrence of *Listeria* in biofilms and analysing the microbiota in a frozen vegetable processing environment

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Listeria monocytogenes, a food-borne pathogen causing listeriosis, is of particular concern for food safety, since the versatile bacterium is known to be able to colonize and survive in food processing environments despite hygiene measures, resulting in potential contamination of food products. In recent years, outbreaks of listeriosis were linked to contaminated frozen vegetables, but knowledge on the presence and behaviour of *Listeria* in frozen vegetable processing environments is very limited.

The current study investigated the occurrence of *Listeria* spp. in a European frozen vegetable environment over a two-year period. Using whole genome sequencing data, in-house clones surviving in the processing environment were identified and the genome content of in-house and transient clones was compared. The stress resistance gene repertoire supported the colonization of the processing environment. Differences in the gene content were MLST-ST specific, yet they did not fully explain the colonization ability of in-house clones when compared to transient clones. Therefore, the presence of *Listeria* in biofilms and the co-existing microbiota was further explored after cleaning and disinfection. Biofilms were detected in 12.7% samples (n=9), of which two were positive for *Listeria*. The majority of *Listeria* were detected on modular conveyor belts, a hard-to-clean niche, suggesting being a major factor for *Listeria* contamination.

Differential abundance analysis using 16S rRNA gene sequencing data showed a higher abundance of ASVs assigned to Enterobacterales (*Enterobacter, Serratia*, Unclassified Enterobacteriaceae) and *Carnobacterium* in *Listeria* positive samples. Surprisingly, several *Pseudomonas* ASVs were less abundant in *Listeria* positive compared to *Listeria* negative samples. *Exiguobacterium* and *Janthinobacterium* were significantly higher abundant in biofilms. We further detected differences in the microbial composition between different room types and between surface material types of sampling sites.

In conclusion, we identified several in- house clones and showed that *Listeria* – as a very versatile organism – has the ability to survive in co-occurrence with different bacterial taxa in various types of niches. Further research is required to unravel the complex ecological interactions within the various niches found in processing environments.

S5-PL02 Exploring antimicrobial resistance and plasmid diversity in the early life gut microbiome

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The escalating global challenge of antimicrobial resistance (AMR) alongside the enigmatic role of plasmids within the human gut microbiome constitutes a frontier for microbial ecology and public health research. This presentation synthesizes findings from two studies



conducted by my group, shedding light on the intricacies of AMR and plasmid dynamics within the gut microbiomes of early life. The first study unravels the acquisition and determinants of antibiotic resistance genes (ARGs) in a cohort of 662 Danish children during their first year, revealing a bimodal distribution of ARG richness influenced predominantly by gut microbiome composition, particularly E. coli. Notably, environmental factors including antibiotic exposure play a significant role in shaping ARG profiles, which in turn correlate with gut microbiome maturity and potential health risks such as asthma. The second study pioneers a novel approach to plasmid analysis in the gut microbiomes of 34 mother-child cohorts, uncovering a previously underestimated plasmid diversity. This exploration not only expands our understanding of plasmid-host interactions but also illuminates the mechanisms through which plasmids enhance bacterial adaptability, especially in infants. Together, these studies offer novel insights into the microbial dynamics of the gut, emphasizing the critical need for innovative strategies to manage AMR and understand microbial gene transfer mechanisms in the context of human health.

S5-ST02 Understanding the association between the bacterial communities of broiler meat and *Campylobacter* for enhanced food safety

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Campylobacter remains the leading cause of zoonosis in humans in



Europe, and poultry is the main source of human campylobacteriosis. The microbial communities on food are important indicators of food quality and safety, potentially influencing the survival of foodborne pathogens like *Campylobacter*.

In a French slaughterhouse, 480 carcasses were collected immediately after chilling during 10 slaughtering days over a 3-month period. *Campylobacter* was quantified by plate counts on CASA agar and bacterial communities characterized by 16S rDNA sequencing, from carcass rinses before (day 0, D0) and after seven days of refrigerated storage (D7). Co-occurrence network analysis were performed using CoNet Software with the Spearman correlation coefficient adjusted to 0.3 and Bonferroni correction. The package R PLNmodels was used to make another co-occurrence network analysis based on the Poisson log-normal model.

The aim is to improve broiler meat safety by unraveling the association between the broiler meat microbiota and *Campylobacter*, and identifying new risk mitigation strategies based on potential interaction within the microbiota.

184 and 96 clusters were identified respectively before and after storage. All of the samples were incorporated in the network analysis, and 75.6% were *Campylobacter* positive and 24.4% negative. At D0, 8 species co-occurred with *Campylobacter* on CoNet, and 37 on PLNmodels, including *Helicobacter pullorum*, *Bifidobacterium pullorum*, and *Barnesiella visceriola* (with respective CoNet interaction weights: 0.435, 0.437, 0.379). This suggests potential carcass co-contamination with intestinal content during early slaughter. *Bacteroides vulgatus* and a *Butyricicoccaceae* family species were negatively correlated with *Campylobacter* on PLNmodels. At D7, *Campylobacter* co-occurred with 3 species on CoNet, including *Lactococcus raffinolactis* and *Pseudomonas sp.*, major genera in refrigerated poultry meat. On PLNmodels, 15 species co-occurred with *Campylobacter*, such as *Falvobacterium antarticum* and *Psychrobacter glacincola*. *Shewanella baltica*, *Shewanella frigidimarina* and *Pseudomonas sp.*, psychrotrophic spoilage bacteria, were mutually exclusive with *Campylobacter* on both CoNet and PLNmodels.

Bacterial species positively correlated with *Campylobacter* on carcasses may indicate its potential presence, while those negatively correlated could represent antagonistic bacteria for controlling *Campylobacter* contamination.

S5-ST03 The pros and cons of tracking the microbial contamination in infant food processing chain through amplicon sequencing and metagenomics

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The targeted sequencing of the 16S rRNA gene's amplicon has been extensively exploited in the last decades to characterise composition and structure of microbiotas in a vast number of habitats, ranging from the human body to the most extreme ecological niches on Earth. Now, more attention is being paid to metagenomics, since it adds pivotal information on microbiome functionality, although it requires higher analytical costs and more advanced computational approaches. The transition from observational studies to practical application of these two omics has begun in medical research, where specific profiles of patient's microbiomes are nowadays linked to their health conditions and the probability to develop diseases. In food science possible applications need to be assessed in diverse food processing plants, in order to be fully understood and thus effectively applied by the industries in microbial control.

To address this, we have profiled the microbiota and the microbiome of an infant food processing plant, for more than a year, through DNA-based amplicon sequencing and metagenomics. A total of twelve productions of infant cereal-based formula were followed and one hundred and fifty samples were collected from raw materials, environment, intermediate and final products. In parallel, the presence of microbial populations of interest has been verified by qPCR assays.

Both amplicon sequencing and metagenomics highlighted high prevalence of *Bacillus* spp. in raw materials and environment, which was confirmed by targeted qPCR detection. The resident microbiome changed over time, with a rapid turnover of major taxa and a fluctuation of communities' biodiversity mainly linked to the incoming of new raw materials, in the presence of a high level of airborne microbial contamination. Greater resolution of taxonomic assignments was achieved by metagenomics compared to the amplicon sequencing approach, but only in samples with high DNA quality and quantity. As to be expected, the hindering effect of non-bacterial DNA was less evident in amplicon sequencing data than with metagenomic data. Alternative alignment strategies were therefore tested to maximise the taxonomic and function assignments of metagenomic data and to detect potential signatures of targeted microbes.

The outcomes underline the need to develop specific approaches for the analysis of the microbiomes in food processing plants.

S5-SF01 Use of shotgun metagenomic for the analysis of shellfish virome: Comparative assessment of the performances of three capture enrichment kits

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Shellfish are filter feeders having the potential to accumulate various



microorganisms, including pathogens, posing risks for consumers. Shotgun metagenomic sequencing is a powerful tool to assess the whole microbiome of shellfish, but the virome component is difficult to analyse due to the small genome size and low concentration of human viruses in shellfish. This study explored the performances of three kits for capture-based viral enrichment and RNA library preparation to sequence viruses relevant to public health, including *Caliciviridae, Astroviridae*, and *Picornaviridae*.

Four wild oyster and one mussel samples, collected in January and February 2021 and 2023 from two areas prone to human sewage contamination in France have been used for the study. For all five samples, digestive tissues were dissected/isolated, as bivalve molluscs harbour the highest concentration of human enteric viruses in their digestive tract. After eluting viral particles and conducting RNA extraction with reverse transcription into cDNA, library preparations were performed in triplicate for each sample using three kits: Twist Total Nucleic Acids Library Preparation EF Kit 2.0 for Viral Pathogen Detection and Characterization (Twist Biosciences, San Francisco, USA), a Roche kit (Roche, France), and Illumina[®] RNA Prep (The Viral Surveillance Panel, Illumina, San Diego, USA). Libraries were sequenced on the Illumina NextSeq 2000 platform, in paired ends 2x150 bp and data were analysed using an in-house Nextflow bioinformatic pipeline.

The results indicated that sequences belonging to the *Caliciviridae* family were better achieved using the Twist's kit, which identified different norovirus genotypes. For the *Picornaviridae* family, the Twist kit showed two viral contigs, while the other kits did not provide any contig. However, for the *Astroviridae* family, even though the Roche's kit generated more contigs, its average coverage was lower compared to the other kits. Additionally, analysis of alpha-diversity at specie-level was significantly higher for the Twist kit (p=0.018), demonstrating this library preparation method outperforms other kits in terms of capability to capture viral richness in the treated biological matrixes.

In conclusion, the effectiveness of the Twist bioscience kit in identifying various virus genotypes underscores its value in detecting viral sequences. The comparison of these kits provides practical insights for future investigations in food virome.

S5-SF02 Deciphering the dynamics of antibiotic resistance transfer through natural transformation in bacterial communities

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Agri-food systems are crucial hotspots for antibiotic resistance diffusion under the One Health perspective. Antibiotic resistant bacteria and



antibiotic resistance genes can enter agri-food ecosystems through wastewater treatment plant (WWTP) effluents released to surface water bodies and used for irrigation purposes. Plants are thus considered a "bridge" connecting terrestrial and aquatic ecosystems with the human microbiome. Natural transformation, one of the Horizontal Gene Transfer (HGT) mechanisms, is of key importance for the antibiotic resistance spread in environmental bacterial communities, especially those inhabiting aquatic ecosystems. This HGT mechanism has been studied in single bacteria populations, using few model strains, thus it is an open question to what extent bacterial interactions can affect the transfer of antibiotic resistance by HGT events in complex communities. In this work, we investigated the effect of environmental bacterial strains on the transformation frequency of Acinetobacter baylyi strain BD413, a model strain for natural transformation, using the plasmid pSEVA431-Gfp as extracellular DNA. We took advantage of a collection of bacteria isolated from surface waters collected downstream a WWTP located in the Cremona municipality, belonging to bacterial genera of interest in the One Health approach and resistant to different antibiotics. Among the collection, we selected the bacteria i) sensitive to streptomycin, the antibiotic used for the selection of the transformant cells and ii) unable to inhibit the A. baylyi BD413 growth according to a dual culture test, therefore suitable to test its natural transformation frequency in a synthetic bacterial community. Furthermore, these bacteria were able to produce exopolysaccharides and to adhere on solid surfaces, relevant traits for biofilm production, and to colonize the rhizosphere of lettuce plants. The performed natural transformation assay showed that the transformation frequency of A. baylyi BD413 significantly decreased of one order of magnitude, in presence of all tested Acinetobacter, Klebsiella and Escherichia environmental isolate. Further experiments will be performed to clarify the mechanisms behind the observed phenomenon. Overall, the data suggests that is pivotal to integrate the effects of ecological interactions occurring in bacterial communities to better estimate HGT occurrence into the environment.

S5-SF03 Meta-taxonomic analysis of poultry and slaughterhouse microbiota: A comprehensive examination of resident microbial communities

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Poultry is among the world's most consumed foods. From farm to fork, contamination is a common occurrence along the poultry processing chain. The final microbial profile of poultry meat, including pathogenic and spoilage microorganisms, is influenced by each step in this process. The consumption of contaminated poultry poses a significant threat to public health, as it is associated to infections caused by foodborne pathogens such as Campylobacter, Salmonella, Listeria monocytogenes and Arcobacter. To reduce clinical cases, it is crucial to comprehend the contamination pathway throughout the food process and characterize the microbiota present in production environments. This understanding is essential for the implementation of appropriate sanitization procedures. This research aims to evaluate the extent to which the skin and ceca of broilers contribute to the indigenous microbiota within a slaughterhouse. Furthermore, it seeks to elucidate how the persistence and distribution of Arcobacter spp. in the environment are influenced by the patterns of crosscontamination associated with these specific sources. Therefore, 16S-rRNA gene-based total DNA sequencing was performed to reconstruct environmental contamination pathways within a slaughterhouse. Broiler neck skin (BNS) and caecum (BC) were sampled during processing, while environmental swabs (SE) were collected from surfaces after disinfection. Meta-taxonomic analysis showed a significant influence of environmental contamination on the microbiota of chicken skin. At the highest taxonomic resolution, the sampling sources showed a distinct composition and distribution of the microbiota at the genus-species level. Arcobacter butzleri emerged as one of the most abundant species and was detected throughout the slaughterhouse, showing a higher prevalence compared to other Campylobacterota. It was uniquely and significantly associated with BNS and SE, while Helicobacter pullorum and Campylobacter jejuni were indicators of BC. Our findings have emphasised the persistence of Arcobacter spp. in a modern poultry abattoir and its establishment as part of the resident microbiota in specific environmental niches. The analysis conducted underlines the significance of early monitoring of food pathogens in the production chain, supported by metataxonomic analysis. Using these detection approaches, the presence of these pathogens could soon be considered an indicator of food safety and quality in slaughtered poultry.



Microbiome diversity and food quality

S6-PL01 Interplay between microbiome and storage conditions in spoilage mitigation

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Microbial spoilage is a major cause of food waste. Food gets contaminated by spoilage microorganism originating from the raw materials and from microbial consortia inhabiting food processing facilities. Several preservation technologies are used by the food industry



to prevent microbial growth and activity thus prolonging shelf-life of manufactured food. Their effectiveness and outcome vary for microbial communities specific for different food types. Ready-toeat (RTE) meals represent examples of complex food composed from different types of materials. This type of food production and consumption is on the growth trend due to consumers demand for convenient and fresh meal with few preservatives. Ready-to-eat meals require no or minimum processing before consumption and are preserved at the chilled conditions with or without modified atmosphere packaging (MAP). Storage temperature violations can happen during the whole production chain including logistics, retail, and domestic storage of a product. In my talk I will show the effect of storage temperature and MAP packaging on the shelf life and dynamic of spoilage microbial population on the example of industrially produced mayonnaise-based potato salad. Four salad batches produced in a three years' time were sampled during storage at different chilling and abuse temperatures. The results obtained by ISO standard microbiological methods traditional for food industry followed by MALDI-TOF identification of the isolates and microbiome analysis based on PacBio nearly full-length 16S rRNA gene and ITS amplicons sequencing were compared. Potential sources of contamination, interactions within microbial communities and possibilities for shelf-life prolongation for the ready-to-eat meal will be discussed.

S6-ST01 Forecasting sourdough aromatic profiles by integrated multi-omics data into a community-wide metabolic network

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Sourdough starters harbor microbial consortia that benefit the final bread's aroma. The complex structure, interaction, and dynamism of this microbiota raise challenges in predicting the fermentation phenotypes. To control fermentation process and meet consumer demands on bread organoleptic profile, ferment manufacturers need to develop tools, able to describe microbial community-wide metabolic pathway ongoing during flour fermentation. Using an integrative multi-omics approach, we aim here to develop a software allowing us to predict the impact of substrate, fermentation parameters and microbial consortia on the production of targeted aromatic molecules in sourdough (Lesaffre), or other applications as in cheese (Bel). In a particular study case, in which we objective the increase in fruity flavour through shorter fermentation time, a preliminary phase has been done with the objectives to define the parameters influencing the most the aromatic profile. 10 different fermentation modalities with 5 variables, tested in biological triplicates across 3 different sampling times have been used, representing a total of 100 unique analytical points. Variables such as flour types (rye or wheat), fermentation temperatures, control of higher pH, bacterial and yeast strain types have been evaluated. Through each fermentation modalities, basic physico-chemical parameters have been metatranscriptomic, reported. Metagenomic, various targeted and non-target volatilomics/metabolomics and agar plat microbial counting have been generated in triplicates for each analytic point and then integrated for metabolic pathway maps reconstruction at the community level. Correlative analysis between aroma compound and variables highlights than pH regulation and flour types negatively influence the presence of organoleptic molecules. Reconstruction of metabolic network at the community scale demonstrate that a spontaneous reaction using protons as co-factor was involved in aromatic molecule synthesis. Also, key enzyme involves in this metabolic pathway were not coding by microbial gene but most likely by flour enzymes using dioxygen as main co-factor. Finally, we noticed numerous genes, express by yeast only, implied in other aromatic compounds synthesis. In conclusion, we decide in a second phase of experience to play with pH regulation, flour composition and yeast inoculation level to maximize the production of aromatic compounds.

S6-ST02 PDO and non-PDO Roccaverano cheeses: Exploring microbiome diversity and correlations

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Roccaverano Protected Designation of Origin (PDO) is a fresh soft cheese produced in the Roccaverano area (AT), using raw whole goat's milk or with the addition of raw whole cow's and/or ewe's milk in a variable ratio.



The research aims to enhance understanding of the microbiome composition of PDO and non- PDO cheeses exploring potential correlations with the production area.

The sampling consisted of 15 Roccaverano cheeses produced exclusively with goat milk from various producers and 15 non-PDO cheeses of similar type from the same area and from large-scale distribution. The DNA was subject to Illumina shotgun sequencing to reveal insights into bacteria and fungi. Microbiota composition was assessed using MetaPhlAn and bowtie2. Metagenome-Assembled Genomes (MAGs) were generated utilizing SPAdes and metabat2.

Lactococcus lactis emerged as the predominant species in PDO and non-PDO cheese groups. Comparable levels of bacterial α -diversity were noted across both groups, while a noteworthy positive correlation was noted between Acinetobacter spp. and Pseudomonas spp. in PDO and non-PDO cheeses, respectively. Conversely, Streptococcus thermophilus was negatively correlated to PDO cheeses (p-value < 0.05). The bacterial β -diversity revealed PDO cheeses clustering closely with certain non-PDO cheeses on the same plot area, suggesting a degree of similarity between PDO and artisanal cheeses. Notably, no significant clustering between PDO and non-PDO MAGs was observed (Roary).

In terms of fungi, the two predominant species identified in both groups were *Kluyveromyces lactis* and *Kluyveromyces marxianus*. Seventy species were correlated with either PDO or non-PDO cheeses, with *K. lactis* demonstrating a positive correlation with PDO (*p-value < 0.05*). Additionally, fungal α -diversity was higher in PDO cheeses exhibiting greater stability across samples compared to non-PDO cheeses, indicating a heightened diversity within PDO varieties.

Despite the selection of cheeses based on their technological similarities, notable differences were observed between PDO and non-PDO, particularly in fungal composition. The next phase of this study will concentrate on volatile organic compounds analysis, integrating statistical studies with sensory analysis data to explore potential correlations with cheese microbiome. This approach promises to shed further light on the intricate relationships between cheese microbial composition and sensory characteristics.

S6-ST03 From science to practice: Bio-preservation for storage improvement – a sugar beet case study

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According to the Food and Agriculture Organisation, approximately one



third of all food produced for human consumption is lost or wasted each year worldwide. This loss occurs throughout the entire food supply chain, including production, processing, distribution, and consumption, with storage being a significant contributor at up to 13%. In particular the sugar sector would benefit from an improved storage capacity, as the prolonged post-harvest storage of sugar beet leads to an increased sugar degradation to invert sugar and the proliferation of microbial pathogens, accelerating decay and resulting in significant yield and quality losses.

One way to increase the storage capacity of food raw materials is through the use of microbes, a process called bio-preservation. Beneficial microbes can outcompete spoilage and pathogenic microorganisms for nutrients and ecological niches, thereby reducing their growth and activity. In this case study, we investigated the microbial communities of good and poor storable sugar beet varieties and characterised a storage capacity-specific microbial community profile. In addition to these scientific results, we isolated bacterial strains from the good storable varieties and characterised their antimicrobial behaviour. One selected strain was then applied onto freshly harvested beet roots of three varieties, which were then stored for about 10 weeks under semi-controlled conditions. This bio-preservation process ultimately led to a significant reduction in storage-related sugar loss in the least storable variety. Although these results are preliminary, they hold promise for a novel practical application of microbes for effective sugar beet storage management.

S6-PL02 Contamination maps as a tool to improve and secure food production in a farm-to-fork strategy

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Food safety regulations in the EU require a comprehensive approach that



encompasses all stages of the food system, from farm to fork. Likewise, using an integrated method allows for the tracking of contamination events throughout this system. Microorganisms are introduced at various processing steps and disseminate within the microbial community downstream along the production chain. To enhance our understanding of dynamic changes in microbial communities depending on processing methods, we investigated different contamination scenarios in beef, pork, and milk processing. We developed contamination maps that facilitate tracing back the origin of microbiota in the final product, to the processing step where contamination occurred during production. We also use such contamination maps as tools to describe the dynamics of contamination along food processing, espcially when natural (=without use of ripening cultures) ripening is envisaged.

In this presentation, we will guide the audience through a hard-cheese-production process, from salting to ripening (180 days). In a series of studies, we characterized the dynamics of microbial changes during ripening, evaluated metabolic activities of microorganisms, formulated selective agar media to isolate the five predominant species that drive ripening, and sequenced their genomes. We identified certain species, harboring an increased number of proteolytic gene clusters. Leveraging these unique microorganisms, as unique, in-house ripening facilitators, we initiated ripening trials in collaboration with food industry. This approach could shorten the ripening time, thereby creating value for the company, while also producing a microbiologically more stable product. In addition, promoting appropriate ripening performance reduces the growth of pathogens such as *L. monocytogenes*, thereby enhancing the product's safety margin.

S6-SF01 Exploration of microbial ecology as a quality marker through its linkage to the geographical origin of spontaneously fermented food matrices: The case of wine, green coffee beans, and cocoa beans

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Agricultural and food products are intrinsically linked to their geographical origin, giving them distinctive characteristics. It has been observed that fermented foods from different geographical regions have different flavour profiles, resulting in differences in the quality of the final product. These correlations have been attributed, among other factors, to the microbiota. However, the exact mechanisms by which microbial distribution patterns influence the metabolites of spontaneously fermented foods remain unclear. Microbial ecology evolves dynamically during different stages of processing, and within wine, green coffee beans, and cocoa beans, individual microbial groups exhibit specific functional roles. Consequently, the composition of the microbiota represents a potential avenue for the identification of geographical origin. The aim of this study was to investigate the microbial communities of three different matrices in order to identify possible links between the microbiota and the geographical origin of these fermented products and to understand how microbial communities could be used as indicators of the origin of the final products. For all the matrices, samples were taken from different regions at different times during the fermentation process in order to study the patterns of the geographical distribution of fungi and bacteria and their correlation with the quality of the final product. For this purpose, the fungal and bacterial communities were studied by high-throughput sequencing of bacterial 16S rRNA and fungal 26S rRNA encoding genes. Chemical analyses of the main secondary compounds were carried out to determine the correlations between regional distribution patterns of microbial communities and their association with the chemical composition. The importance of this study is to understand the potential use of microbiota as an indicator of origin and as a predictor of the metabolite composition of the final product, to be able to use them as quality markers.

S6-SF02 Dynamic interactions between bacterial and fungal communities on vacuum packaged beef: A comprehensive longitudinal microbiome analysis

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Understanding the microbiome dynamics on vacuum packaged beef is crucial for optimizing food safety and shelf-life. This study is based on a longitudinal analysis, sampling nine pieces of beef over 85 days at ten timepoints each, to illuminate potential interactions between bacteria and fungi within microbial communities. Such interactions, documented across various environments such as soil, gut, and cheese, prompted the hypothesis that fungi might similarly influence microbial communities on meat. This initial phase of research aims to delineate the microbial populations on vacuum packaged beef and identify any potential inter-species interactions. Beyond characterizing these communities, this study sets the stage for subsequent experiments involving microbial consortia challenged with selected fungal isolates to assess their impact on microbial dynamics.

Utilizing a comprehensive array of microbiological counts, 16S and 18S amplicon sequencing, and qPCR, we precisely quantified microbial populations, isolated strains of interest, and depicted growth curves for dominant amplicon sequence variants (ASVs). Our approach, refined by the integration of spike-in cells, facilitated the determination of absolute abundance data, offering an in-depth chronological perspective of microbial community shifts. While the full spectrum of bacterial and fungal interactions remains to be clarified, our preliminary findings hint at intricate relationships that could subtly affect meat preservation methods. This research not only enriches our grasp of the vacuum packaged beef microbiome but also establishes a foundational framework for ensuing inquiries into microbial interplays and their implications for food safety.

S6-SF03 Substrate drives densely connected microbial community assembly across diverse traditional fermented foods

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The beneficial effects of fermented foods for human and planetary health is increasingly being appreciated. Fermenting food is emerging as an alternative approach in food preservation compared to current industrial practice of preserving food by highly processing them and adding chemicals that are detrimental to human health. But throughout history, indigenous peoples across the world have used fermentation as an instrumental technique for food preservation, improved digestibility, and flavour enhancement. Although there is a plethora of uniquely fermented foods across the world, only a limited number of them are commercially produced. Furthermore, industrial fermentation primarily depends on a handful of starter microbial strains while traditional approaches rely on naturally occurring microbes to spontaneously ferment foods. The process of microbial community formation in traditionally fermented non-European foods remains understudied. Here, we characterized the bacterial and fungal communities in diverse plant and animal based fermented foods from Nepal, South Korea, Ethiopia, and Kazakhstan traditionally prepared for household consumption. Traditional fermented foods are reservoirs of diverse and densely interconnected ecosystems of bacteria and fungi that reside in biofilms on the food surface. In addition to the canonical fermenters - lactic acid bacteria (LABs), Bacillales, and Saccharomycetales (yeasts) - that are commonly used in commercial fermentation, these foods comprise of additional microbes that contribute to fermentation process or in aesthetics (taste, flavour) as well as environmental microbes. Environmental bacteria differentiate the fermented foods across geography but substrates shape the bacterial community as well as bacteria-bacteria and bacteria-fungi interactions in traditional fermented foods. Plant-based ferments harbour bacterial communities that putatively degrade carbohydrates while animal-based fermented foods contain bacteria enriched with potential to degrade proteins and lipids. Our results indicate that fermented food microbiome community assembly is a highly dynamic process and underscores the need to develop an ethical and equitable framework for investigating the microbial communities of traditional fermented foods to maximize its potential benefits for human health.



The edible microbiome

S7-PL01 The edible microbiome: Rethinking the concept of "You are what you eat"

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The link between food and health has been recognised since ancient times, and in the last century, dietary guidelines and nutrition programs became very common. Initially, these guidelines and programmes focused on the nutritional components. As the awareness and



understanding of the gut microbiome and its effects on human health and well-being increased, the effect of food on the composition and functioning of this complex system was also progressively addressed.

The edible microbiome is a constituent of many foods we eat (e.g. raw plants, different fermented foods) and thus an integrative part of our diets. However, its interactions with the gut microbiome or the consumer are still not fully understood. In this presentation, the first insights and the importance/implications of this emerging research field will be discussed.

S7-PL02 The edible microbiome and human (gut) health

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Diet is considered one of the main factors of exposure because of its significant impact on gut microbiome and human health. While exposure to diet is well studied, the edible plant microbiome, defined as microbes



residing in raw-eaten vegetables, fruits, and herbs that we consume, has been largely ignored in this context. Raw-eaten vegetables, fruits, and herbs carry trillions of microorganisms with diverse genetic reservoirs during each meal. To understand if and how fruit and vegetable associated bacteria contribute to overall gut bacterial diversity we reconstructed metagenome-assembled genomes from 156 fruit and vegetable metagenomes to investigate the prevalence of associated bacteria in 2,426 publicly available gut metagenomes. The microbiomes of fresh fruits and vegetables and the human gut are represented by members in common such as *Enterobacterales, Burkholderiales*, and *Lactobacillales*. Exposure to bacteria via fruit and vegetable consumption potentially has a beneficial impact on the functional diversity of gut microbiota particularly due to the presence of putative health-promoting genes for the production of vitamin and short-chain fatty acids. In the human gut, they were consistently present. Host age, vegetable consumption frequency, and the diversity of plants consumed were drivers favouring a higher proportion. Evidence that fruit and vegetable-derived microbes could be found in the human gut and contribute to gut microbiome diversity supports the importance of the edible microbiome.

S7-ST01 Microbes on the menu: Investigating rocket salad as a vehicle for microorganisms for the human intestinal ecosystem

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The *Microbial Deprivation Hypothesis* posits that early-life exposure to harmless microorganisms is essential for proper immune system



development, reducing the risk of autoimmune diseases and allergic disorders. Industrialization plays a significant role, with reduced contact with rural environments and animals, as well as extensive sanitation, leading to decreased exposure to beneficial microorganisms. The composition of food is also pivotal in modulating intestinal microbial diversity. While research has elucidated how fermented foods contribute to microbial diversity in the human gastrointestinal (GI) tract, less is known about unfermented foods, such as raw vegetables. Contextually, we studied ready-to-eat rocket salads (RS) to test the hypothesis that cultivation methods may affect the taxonomic diversity of RS-associated microbes. Furthermore, we investigated the ability of RS-associated lactic acid bacteria (LAB) to survive GI transit.

Bacterial load was comparable among land-based cultivation methods but generally lower in vertically farmed RS. Notably, viable LAB were absent in vertically farmed samples, while land-based samples harbored LAB between 2 and 5 log₁₀ colony-forming units per gram (CFU/g). Most LAB isolates in RS were taxonomically assigned to genera *Leuconostoc* and *Weissella*. Metataxonomic analysis highlighted higher evenness and richness in land-farmed RS compared to vertically farmed ones. Furthermore, β -diversity analysis revealed lower inter-sample diversity in land-farmed salads compared to vertically farmed ones. In vitro experiments demonstrated the ability of RS-associated bacteria, especially LAB, to survive the human GI tract. Human intervention studies confirmed the ability of RS-associated *Weissella* spp. to survive GI transit. Viable RS-associated LAB were found in volunteers' faeces only after consuming RS with a higher initial load (4.1 log₁₀ CFU/g), not with washed RS (no detectable LAB), or RS with a lower load (2.3 log₁₀ CFU/g), while not affecting the total LAB viable count in faeces. These findings underscore the potential for raw vegetable-associated microbes to survive the human digestive tract and persist within the gut, potentially contributing to the shaping of the human intestinal microbiome.

S7-SF01 Unravelling the fermented olives microbiome: Bacterial diversity, function and its importance for human health

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Fruits and vegetables are an essential component of our diet and contribute to our gut microbiome, thereby directly impacting our health. Raw fruits and vegetables are especially beneficial as their consumption leads to the transfer of indigenous fruit and vegetable microbiome to our gastrointestinal tract. Fermented foods such as olives contain high populations of Lactic Acid Bacteria (LAB) and are a valuable source of microorganisms, alongside raw foods. The conditions of pickled green olives might support the dominance of highly diverse LAB species. Based on genomic data, olives could be a reservoir for novel LABs with probiotic properties. However, our understanding of the microbial composition of fermented olives remains limited. Our study aims 1) to evaluate the bacterial diversity and community structure of the olive microbiome and 2) to identify their putative function related to human health. Olive samples from different countries and producers will be examined in this study. We will use a polyphasic methodology, including independent and culture-dependent experiments, to evaluate the bacterial diversity, community structure, and function of olives. We will use amplicon sequencing of the bacteria and lactobacilli 16S gene V4 hypervariable region to determine the bacterial and lactobacilli community structure. Quantitative real-time PCR will be used to estimate the total bacterial and lactobacilli abundance. Furthermore, we will isolate bacteria from olives using various media and subject them to functionality assays related to probiotic and survival properties, including the presence of biosurfactants, antagonism against human opportunistic pathogens, and bile salt resistance. The study will provide valuable information on the diversity, community structure, and function of the olive microbiome, laying the groundwork for future targeted intervention studies using olive. We will also conduct a storage experiment to determine the impact of different storage practices on the olive microbiome. Olives will be stored in glass jars and vacuum-sealed plastic bags at various temperatures, and samples will be collected for amplicon sequencing of the bacteria 16S gene V4 hypervariable region. The results of this part will provide critical insights into the potential implications of different storage practices on the olive microbiome and its potential health benefits.



Microbiomes for improving health and well-being

S8-PL01 From the microbiome to the electrome: Implications on the gut-brain axis

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While it is clear that bacteria in the gut and neurons in brain communicate (the microbiome-gut-brain axis, MGB), many of the fundamental questions in this field are wide-open. Nowadays, the scientific community has focused on indirect pathways of connection between (gut) bacteria



and (brain) neurons, which are executed through metabolites and signals, but much better understanding is still required before therapeutic and nutritional-focused tools can be developed to target the MGB axis. There is a pressing need to reveal the actual mechanisms of action, which may go beyond what is already known, and can only be derived from radically new methodologies and a multiintegrative "top-down" scientific approach that provide a holistic understanding of the bidirectional communication between neurons and bacteria. To address this gap, we are developing the first integrated electrical-optical Brain-Bacteria Interface (BBI), a multi-site stimulation and recording platform specifically suited to extract information in real-time across these highly diverse biological entities. Using real-time optical (calcium signaling) readouts, morphological approaches and transcriptomic analysis we show that neurons react to the presence of bacteria, and that this response is influenced by specific properties of the bacterial cells. The extraction of information content from signaling between neurons and gut bacteria, and the establishment of a novel interface between synthetic biology, biophysics, information science, and molecular physiology will produce new knowledge of both basic and applied impact, bridge a capabilities gap that establishes a new direction for the field, and serve as an enabling technology for biomedical, commercial and other contexts to infer a food-based intervention regime for a desired outcome in mental wellness.

S8-PL02 Harnessing microbiome data to create the next generation of fermented foods

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A tremendous variety of fermented foods are produced by all societies globally. However, there are still many fermented foods that have yet to undergo in-depth microbiome analysis to reveal the diversity of species and strains present therein. An even smaller subset of fermented foods



have been the focus of pre-clinical/clinical studies. Despite this, the studies that have taken place show a huge untapped potential. This potential can be achieved through the creation of communities of fermented food microorganisms designed to capture key health promoting, and other, features of fermented foods in a manner that also ensures that highly consistent products can be generated at scale.

S8-ST01 Nutritional iron deficiency effects on microbiota-gut-brain axis

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Iron deficiency (ID) is the most frequent nutritional deficiency in the world. While it is clear that nutritional ID has an impact on the metabolism and cellular biochemistry of gut bacteria, the potential implications in the microbiota-gut-brain axis (MGB) are poorly understood. Indeed, dysbiosis in the gut microbiota, observed in early-life ID, is associated with neurodevelopmental impairments, including autism spectrum disorder and attention deficit hyperactivity disorder. Understanding the effects of ID on bacterial signaling offers avenues for targeted interventions to mitigate neurodevelopmental risks in iron deficient populations. In this study, we investigated the responses of two representative bacterial species of human microbiota, Escherichia coli (E. coli) and Limosilactobacillus reuteri (L. reuteri), to experimental conditions of ID. Using the iron chelator 2,2'-Bipyridyl (BP), we developed a reliable method for the creation of in-vitro ID conditions on bacteria cells. Then, we assessed and mathematically modelled their growth and cultivability, and we explored the bioelectric profile (electroma) of these bacteria as a potential way of communication with host neurons, using the voltage-sensitive fluorescent dye DiBAC4(3). Our findings revealed that E. coli and L. reuteri behave differentially in response to ID in their culture environment: whereas L. reuteri showed no alterations in growth dynamics neither cultivability when treated with BP, E. coli showed clear decreases in the growth rate and cultivability when affected by ID. Interestingly, only E. coli demonstrated an altered bioelectric profile under conditions of ID, characterized by increased depolarization of cells. Our observations underscore the heterogeneity of bacterial responses to iron deficiency and highlight the complexity of interactions within the gut microbiota. Understanding such variability is crucial for deciphering the role of microbiota in health and disease, particularly in conditions associated with nutritional iron imbalance and neurological disorders.

S8-ST02 Fungi used in food processes with potential probiotic effects on gut inflammation

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Many strains have been used and selected by the food industry for their capacities to ferment, produce flavours, or produce heterologous molecules. Very little is known about the diversity of foodborne filamentous fungi and yeasts and their potential effect on gut microbiota and gut health.

We initiated a complete characterization of 10 filamentous and yeast strains belonging to ten species with a long history of safe use in food with a focus on their capacity to protect against gut inflammation using *in vivo* and *in vitro* approaches. We used an *in vivo* model of DSS-induced colitis in mice mimicking the different symptoms of the human ulcerative colitis.

Most filamentous strains had no strong effect *in vivo* and very little *in vitro* but were only tested in their spore forms.

However, for the yeasts, *Cyberlindnera jadinii* and *Kluyveromyces lactis* living cells showed a clear reduction in mouse sensitivity to colitis *in vivo*. Interestingly, we observed that *C. jadinii* had the capacity to survive the transit in the gut, while *K. lactis* did not. We demonstrated that *C. jadinii* was unable to efficiently adhere to epithelial cells and did not survive more than 24 to 48 h in the gut. Transcriptomic analysis using NanoString[®] technology suggested a potential role of IL-8 through Mif and Fkbp5 in the effect of *C. jadinii* on the immune system. Bacterial and fungal microbiota characterization showed a modification of both microbiota after *C. jadinii* treatment, with a significant increase in positive microorganisms and a decrease in pathobionts.

Altogether, these data suggest that food born fungal strains might have potential as probiotic strains to fight against inflammation in the gut but further studies are needed to widen the diversity of strains tested and understand the mechanisms by which these strains act on gut health.
S8-ST03 Intervention study on the effect of fermented vegetable consumption on gut microbiota for three various focus groups

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It is known that fermentation improves the nutritional properties of vegetables, enriching them with antioxidants and vitamins or increasing



their polyunsaturated and short-chain fatty acids content. However, fermented products also contain living bacteria that can support the immune system: offering protection against harmful inflammatory bacteria and producing signalling molecules that help regulate the immune system.

In the current intervention study, we aimed to investigate the effect of fermented food consumption on general health parameters with a particular focus on gut microbiota. We divided the participants into three groups. The first group consisted of individuals who had taken prescribed antibiotics in the last six months. The second group was made up of people who experienced constipation, and the third group was a control group of healthy individuals. We excluded people with serious gastrointestinal issues, pregnant and breast-feeding women, individuals with extremely low BMI (less than 18,5) and those younger than 18 years.

In addition to their regular diet, participants were offered fresh vegetables, including kohlrabi, carrot, and white cabbage, followed by fermented vegetable intakes such as sauerkraut, kimchi, and fermented carrot. The first three weeks the participants consumed fresh vegetables, followed by a two-week washing-out period and three weeks of fermented vegetable intake. To gradually modify their diet, we began with an additional 50 grams per day of vegetables in the first week, followed by 100 grams in the second week, and 150 grams in the third week. This gradual increase was particularly important for fermented vegetables, which can cause discomfort and bloating. This study design allowed us to distinguish the effects of increased vegetable consumption, with a specific emphasis on the benefits of fermented vegetables. We monitored the participants' general health parameters (weight, BMI, visceral fat etc.) as well as their gut microbiota composition (by 16S rRNA sequencing) before and after each cycle, collecting data from 5 time points for each individual. Additionally, dietary and common health self-reporting questionaries were analysed. The study is ongoing and the comparative analyses between the fermented/non-fermented vegetable consumption inside each group and to the control healthy group will be performed.

S8-SF01 The application of High-Resolution Nuclear Magnetic Resonance (HR NMR) in metabolomic analyses of meconium and stool in newborns - the MABEL project: A preliminary study

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The microbial population living in our intestines plays a key role in several



metabolic, nutritional, physiological, and immunological processes. It is known that infant gut microbiota composition has both horizontal transmission delivery and environmental conditions and a vertical one, from mother to child, related to how the infant is fed (breastfed or infant formula). Detailed information on the composition of meconium and faeces from newborns may help predict the most prevalent and hazardous conditions affecting pregnancies, mothers, and babies, including pre-term birth, preeclampsia, and gestational diabetes mellitus for example. This work aims to demonstrate the feasibility of the whole High-Resolution proton Nuclear Magnetic Resonance (1H HR NMR) procedure in metabolomic analysis in preterm newborns. Thus, multiple samples of meconium and stool were collected from three pairs of premature twins and their metabolite profiles were acquired and exploited by combining the NMR technique with univariate and multivariate analysis. The analysis showed that an impact on the metabolite profile was visible concerning both the sex of the newborns and the couplet of origin. Most of the variation between twin couplets was seen with butyric acid concentration in meconium/stool samples. Despite the low number of samples, the described NMR procedure showed to be a suitable approach to evaluate the similarities of the molecular profiles of different samples, offering a non-invasive and informative approach to understanding the metabolic and nutritional status of preterm infants. Future metabolomic analysis should be supported by microbiome analysis, such a multi-omic approach will provide a more complex view of the development of preterm newborns.

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S8-SF02 Role of fermented oat drink and fermented milk compared to freeze-dried cells in the survival of the probiotic strain *Lacticaseibacillus rhamnosus* CRL 1505 in human gastrointestinal transit

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Since the first observation in the early twentieth century of the positive impact on human health of the consumption of lactic acid bacteria through fermented milk, the role of probiotics was well investigated together with protective role of the food matrix. Nowadays, the probiotics are also commercially available in plant-based formulation, like fermented cereals or vegetables for facing the limitation related to milk protein allergies, lactose intolerance or ethical consideration about the sustainability of milk production.

In a crossover intervention study involving twenty healthy subjects, the ability to survive aKer the gastrointestinal transit of the probiotic *Lacticaseibacillus rhamnosus* CRL 1505 (1x10⁹ CFU per day) was evaluated when administered through fermented milk, fermented oat-based drinks or lyophilized cells. The recovery of the probiotic strains in the faecal samples was evaluated by culture-dependent methods integrated with molecular methodologies employing strain and species-specific primers. Additionally, absolute quantification via quantitative PCR of the probiotic strain and 16S rRNA profiling with Illumina sequencing were performed. The results showed that the survival of the probiotic strain assumed via the oat-based drink and fermented milk was significantly different compared to the lyophilized cells, observed after 3-4 days of consumption and at the end of the treatment. The fermented beverages also resulted in a higher amount of probiotic retrieval in the subjects. The results obtained by the 16S rRNA profiling confirmed the positive role of the fermented beverages as probiotic delivery vehicles.

In conclusion, the data obtained in this work confirmed i) the ability of the probiotic CRL 1505 to survive after the gastrointestinal transit and ii) the well-known protective effect of fermented milk on the probiotic strain. These data could support the possibility to use plant-based matrices as equally effective vehicle for probiotic survival through the gastrointestinal tract.

S8-SF03 Impact of an 8 weeks intervention with orange juice enriched with probiotics and vitamin D on the diet of volunteers at high cardiometabolic risk

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Dietitian nutritionists usually advise people at high risk to avoid consuming foods or beverages with high sugar content such as commercial fruit juices. However, nowadays several researchers report that the moderate juice consumption may be beneficial. To this respect, this work focused on the impact of consumption of orange juice (control) or orange juice enriched with probiotics (Lacticaseibacillus casei Shirota and Lacticaseibacillus rhamnosus GG) and vitamin D (Funjuice) on the health of volunteers at high cardiometabolic risk. From a total of fifty volunteers, twenty-six volunteers were randomly assigned to consume the Funjuice, while the rest of them (twenty-four) consumed the control juice. The volunteers were advised to consume the juice daily for 8 weeks. Every week their body weight, waist and hip circumference were recorded. Before and after the 8 weeks intervention, blood tests were performed and stool samples were collected. Results showed that, the body weight of the volunteers who consumed the Funjuice, was reduced. Microbiota analysis of stool samples showed that at the end of interventions the bacterial diversity of both groups was similar. However, the relative abundance of Megasphaera was increased in the case of Funjuice consumption compared to control. The genera Megamonas and Lachnospira were increased in the case of control, while a lower abundance was detected after 8 weeks intervention with Funjuice. The opposite phenomenon occurred in the case of Coprococcus, where its abundance was decreased in the case of Funjuice and increased in control. Further analysis of the correlation between sequencing data with the tested indicators will give a better insight on the impact of the enrichment of orange juice with probiotics and vitamin D at high cardiometabolic risk volunteers.

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Circular food systems for microbiomes improving animal, human and environmental health

S10-PL01 Circular food systems for microbiomes improving animal, human and environmental health

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Based on analytical tools and insight in composition, function, and role of microbiomes in the food system, concrete steps forward can be taken: Innovative improvement of gut health by making food ingredients and new types of feed from circular use of plant residues. Hereby contributing



to climate change mitigation and to making more room for biodiversity and not the least to improved health of man and animals. Transforming residues from food-processing into gut-healthy ingredients: Fiber-rich residues can be converted into gut-healthy food and feed, by enzyme treatment, cutting the hemicellulose fibres into e.g. short xylan oligoes (XOS). Such short oligoes have been documented to have beneficial prebiotic effects on the microbiome; strengthening the healthy part, hereby outcompeting the unhealthy part of the microbiome. This concept of producing gut-healthy food and feed is not only for plant residues. Another prime example is the Green Biorefinery, using e.g. grass as feedstock. Here the prime product is proteins, but strengthening the commercial viability of the Green Biorefinery significantly is possible if also valorising the hemicellulose (producing Xylan Oligoes for microbiome health). Another approach to convert food processing residues into gut-healthy ingredients is by fermenting the residues. Case: Co-fermenting rape seed cake with seaweed, using lactobacteria. Hereby a triple benefit is achieved: Prebiotics, produced by the enzymes of the fermenting bacteria; probiotics from the LAB; and anti-inflammatory effect of metabolites produced during fermentation. To improve the gut-health effect of food and feed it is imperative to develop new tools for predicting the function of the common "microbiome-interaction-secretome". Description: How the CUPP tool was used to elucidate the change of live-stock feed (less grass and more starch) was correlated to reduced methane emission; and to how change of diet led to change in enzyme secretome of the rumen microbiome. In vitro fermentation using fresh microbiome fluid was used for discovery of feed ingredients with methane reducing effect. It was discovered that chicory and fennel significantly reduced methane gas production. This result is applicable in both conventional and organic production; winter feeding as well as summer grazing; mixing such flowering plants with the grass. The next step is to see if bitter-salad plants, typical for the Mediterranean diet, could also improve gut health in humans?

S10-PL02 Use of microbes to produce more climate friendly food and feed

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The growing population on planet earth is increasing the demand for animal derived proteinacious food sources (meat, milk). Unfortunately, animal derived protein comes with a price of high greenhouse gas emissions pr. gram of protein produced. There is no easy fix to this, but



improving feed conversion efficiency, reducing ruminant methane emissions or increasing consumption of plant-based meat substitutes may all be part of the solution. In the present talk three examples will be given highlighting the potential within each of these areas. 1) Lactic acid bacteria fermented rape seed and seaweed feed supplements alter pig gut microbial ecology, resulting in lowered pathogen load and improved production parameters among production animals. 2) High methane emitting cows have a ruminant microbiome distinct from low methane emitting cows. Interestingly, transfer of ruminant virome content from low methane emitting cows to an *in vitro* rumen system inoculated with ruminant content from high emitting cows reduces the rate of methane production highlighting the potential for reducing methane production via ruminant microbiome manipulation. Finally, and by far the most efficient way of reducing greenhouse gas emissions from the food we eat is to increase consumption of plant-based protein sources. One limitation is the relatively low levels of essential amino acids like methione in many plant-based food sources. We are investigating *Bacillus*-based fermentations as a tool to improve methionine content plant-based matrices with promising results which will be presented as the third example.

S10-ST01

Exploring soil biome complexity: Regional impacts of land management and soil degradation

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Soil biodiversity is crucial for supporting plant yield and other ecosystem services in food production systems. However, unsustainable soil management practices can impair soil biodiversity, leading to an alarming decline in soil functions that are at the base of ecosystem services. This calls for using more sustainable soil management practices, as acknowledged by EU action plans for increasing organic agriculture, as part of the European Green Deal, the Farm to Fork and Biodiversity strategies (COM/2021/141). However, soil organisms could respond differently to sustainable soil management, depending on the biogeographical context and on the severity of initial soil degradation. To better understand this, the SOILGUARD consortium sampled a network of 233 sites across ten European and international regions, including three land use types: arable fields, grasslands and forests. Sites spanned three soil degradation levels, as well as conventional and alternative soil management practices. We quantified abundance and community composition of soil bacteria, fungi, nematodes, mites and springtails, and the community composition of protists and annelids, then analysed soil food webs and co-occurrence networks. The positive impact of sustainable soil management on soil biome assemblages depended on the extent of soil degradation and on the type of organism, suggesting that there is no one-size-fits-all solution for restoring soil biome complexity.

S10-ST02 TRIBIOME: Developing new circular tools for wheat microbiome modulation as an innovative contribution for a sustainable, healthy, and resilient food production system

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The potential of microbiome diversity is gaining recognition both in agri-food systems and society. Revealing the functionality of plant-microbe interactions will lead to a better understanding on how crops can benefit from microorganisms, as well as provide new microbial solutions, like biofertilizers, biostimulants or biopesticides, as urgently required alternatives to their chemical counterpart in EU agricultural practices. In this sense, the TRIBIOME project aims to deep the knowledge of soil-plant microbiome interactions and how they vary in different abiotic and biotic stresses, as well as developing bioaugmentation and biofortification technologies to address their modulation, upgrading food quality and positively influencing the microbiomes of both animals and humans. During this work, a pool of over 150 Plant-Growth Promoting Microorganisms has been isolated from wheat root and soil samples (BBCH 51-57) of different climatic, cultivation zones and countries (Spain, Italy and South Africa), following dilution, incubation and agar plating procedures. Bacillus sp. and Pseudomonas sp., isolated from Triticum durum (Spain) and Triticum aestivum (Italy) root samples, respectively, were selected according to their interesting activities, including: resistance to high salt concentration and high temperature, ability to grow at different pH values and in presence of heavy metals, and capability of producing siderophores, fixing N2 and mobilising organic PO₄³⁻. Bacillus sp. and Pseudomonas sp. also showed high cell viability in growth kinetic studies when cultured in LB medium, reaching 1.3x10⁸ and 4.1x10⁹ colony forming units per mL, respectively, which makes these isolates promising candidates for bioproduction through circular economy-based processes. Further studies will test the suitability of these microorganisms for (i) cultivation from second-generation media obtained from wheat residues and (ii) formulation into end-products to be validated in wheat through pot assays and field trials. Throughout this zero-residue circular approach, TRIBIOME's results will provide new available tools and predictive models for cereals cropland microbiome modulation and will further support the transition to a novel and resilient food production system.

S10-SF01 Utilizing indigenous agricultural soil microbiota for the management of *Listeria monocytogenes* in Indian arable land

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Listeria monocytogenes is becoming increasingly concerning as a pathogen, with numerous deadly outbreaks worldwide linked to the



consumption of contaminated fresh food. According to the CDC, it ranks as the third most common cause of death from foodborne illnesses in the United States. This bacterium is found in the gut microbiota of farm animals, including those used in organic farming where their faeces are often used as manure. This practice may lead to the transmission of *L. monocytogenes* to plants, vegetables, and other farm animals, resulting in human infections upon consuming contaminated produce. Despite recent cases of listeriosis in various parts of India, there has been limited systematic surveillance of soil and compost for this pathogen. Additionally, research on the use of plant growth-promoting rhizobacteria (PGPR) against human diseases, including Listeria, is scarce. To address this gap, soil and compost samples were collected from 15 different locations across India, followed by surveillance for *Listeria* spp. using both culture-dependent and culture-independent techniques were employed to detect these bacteria. A SynCom consisting of 11 strains of PGPR, exhibiting diverse plant growthpromoting and biocontrol activities against *Listeria monocytogenes*, was developed. The mode of action of the SynCom is being investigated. The results of this study not only confirmed the prevalence of *Listeria* species in Indian soil, but also highlighted the potential of native soil microbes in offering an environmentally friendly approach to mitigating human pathogens in agricultural ecosystems.

S10-SF02 Supplementation of agro-industrial by-products in animal feed towards well-fare and sustainability: Case study of sugarcane bagasse lignin

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Animal feed is a crucial factor in the health and well-being of livestock animals. Nutritional strengthening through diet is essential for animal performance and welfare, especially with the limitation of antibiotic use in livestock animals' diets. Searching for valuable feed additives among by-products is a way to promote a circular economy while maintaining functional diets. Many agro-industrial by-products are sources of bioactive compounds that can be extracted and used in feed fortification, modulating the gut microbiota to promote the host's health. Sugarcane bagasse (SCB) is an undervalued residue, containing several interesting bioactive compounds, such as lignin, that can be extracted and used on animal feed.

A case study was performed to evaluate if SCB lignin could be used as a functional ingredient in chicken feed, assessing possible effects on the animal's cecum microbiota, and performance. A total of 108 chicks were separated into 2 groups, fed either a basal diet (BD) or a basal diet supplemented with 1% SCB lignin (BD + SCB lignin), both in mash form. Bird performances, bacterial cecum microbiota, and cecum volatile fatty acids were evaluated.

In this study, SCB lignin increased cecum acetate and butyrate and reduced cecum *Enterobacteriaceae*, results that can be seen as positive health-indicating markers. There was no impact on animal performance.

These results show that lignin has a beneficial impact when supplemented to mash feed diets while coping with sustainable practices, proving the potential for by-products to present themselves as eco-friendly alternatives to feed additive supplementation.

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Microbiomes for plant fermentation

S9-PL01 Plant based fermented foods for healthier diet, addressing nutritional challenges through tradition and innovation

Christèle Humblot

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A strong advocacy to consume less meat and more plant-based foods is necessary for sustainability and improvement of the food system in an environment friendly way. Furthermore, plant-based foods can play a



vital role in the prevention of different diseases such as cancer, cardiovascular disease, or diabetes. The high nutritional density of plant foods, attributed to their rich content of dietary fibre, antioxidants, and vitamins, further underscores their importance in a healthy diet.

Among plants, cereals are of particular importance since they are staple foods all around the world and thus contribute to the coverage of different macro and micronutrients requirements. Nevertheless, they are also lacking of essential nutrients such as some essential amino acids or vitamin B12. Legumes on the contrary, are consumed in very low amount in Europe, despite their high nutritional interests since they are a rich source of proteins, with a good essential amino acids profile, which can complement cereals. Though, they contain compounds such as alpha-galactosides, responsible for abdominal pain. This can partly explain their low consumption rate in Europe, far from the 50 g recommended per day for healthier and sustainable food systems.

Fermentation can enhance the properties of plant-based foods. There is evidence that fermented traditional products show improvements in nutritional quality and health-related properties compared to their non-fermented counterparts. Of the vast fermented cereal-based foods existing around the world, only a few have been deeply studied and description of actors of fermentation and effect of fermentation on nutritional quality are incomplete. Data on the effect of fermentation on legumes are even rarer, with the exception of soy.

Many attempts have been realised on the use of microorganisms selected for their health or nutritional functions with various outcomes. For example, the reduction of alpha-galactosides in soy has been effective, as well as the production of vitamin B during the fermentation of different cereals. However, employing complex microbial ecosystems that combine multiple strains to amplify desired health effects in food is less common.

This presentation will provide an overview of both the successful stories as well as of the bottlenecks, which are important to tackle, in order to achieve simultaneous health and nutritional benefits in the plant fermented food.

S9-PL02 Exploring the metabolic labyrinth of lactic acid bacteria in the microbiome of fermented plant foods: toward a shift in perspective from the individual to the global

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Even though lactic acid bacteria are only a small part of the plant autochthonous microbiota, they represent the most important players capable of promoting significant changes in the health properties of



plant-derived foods. Due to the variety of plant chemicals and possible bioconversion pathways, , plant fermentation is like a metabolic labyrinth undertaken by bacteria.

The success of these paths is linked to the adaptive growth and survival of lactic acid bacteria. In recent years, the panel of various interacting omics approaches has only partially unravelled the specific traits of lactic acid bacteria to adapt to plants. The winding metabolic pathways involve several plant secondary metabolites, among which phenolics and fatty acids are the most exciting for researchers.

Phenolics metabolism by lactobacilli has been somewhat elucidated, but comprehensive information on the contribution of specific enzymes within the microbiome of fermented plant foods is still scarce. Recent pioneering studies have also shed light on the lipid metabolism of diversified lactic acid bacteria and opened up new perspectives on the processing of high-fat plant matrices. Some unconventional microbial species emerged for lipolytic activities and the ability to release hydroxy- and epoxy-fatty acids, in addition to the better-known lactobacilli. These metabolic traits were strain-dependent, as different capabilities were observed in strains belonging to the same species. Although it is reasonable to complete the deciphering of the mechanisms regulating these secondary metabolites at the level of pure cultures, it is even more urgent and worthwhile to dissect these pathways at the level of metacommunity to enable the optimal design of complex microbial processes. Indeed, fermented foods are the result of complex microbial consortia, likely undergoing a long fermentation that reflects a distinctive microbial succession over time. Therefore, it is difficult and restrictive to reason about the role of the individual microbial player.

Obviously, understanding and managing the metabolic labyrinth in the context of mixed cultures is even more challenging, as there are more variables and players involved in regulating such intricate metabolic paths. To address this new challenge, it is necessary to correlate the profile of secondary plant metabolites in fermented matrices with microbiome specifications. The current framework is still very fragmented, but its completion will allow the development of effective targeted fermentation strategies.

S9-ST01 Ancient DNA insights into 7th century Tibetan barley beer brewing from Samdzong, Upper Mustang, Nepal

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Located some 4000 meters above sea level, in the Kali Gandaki river valley of the Upper Mustang region of Nepal, lies the sky cave of Samdzong.



Archaeological excavations conducted between 2010 and 2012, revealed several tombs, among which the tomb 5, which contained human remains and cultural artifacts associated with food and beverage production. Among these artifacts, copper vessels, dated to the 6-7th century CE, are thought to be associated with the brewing and consumption of chhaang, a typical Tibetan barley beer. After conducting DNA extraction in a dedicated ancient DNA cleanroom facility, we shotgun and target captured and sequenced these samples, to check both for plant and microbiome composition. The microbial signal present in these samples revealed an assortment of microbes associated with alcoholic fermentation, while the plant DNA matched with wheat and barley reference genomes. After reconstructing the genomes of some of these microbes, we demonstrate their ancient origin, and show that their gene composition is consistent with alcoholic fermentation. Taken together, these findings support the hypothesis of chaang production in the Upper Mustang region in the early 7th century CE.

S9-SF01 Understanding taxonomic diversity and functional signatures of fermented vegetables microbiome

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Fermented vegetables are becoming increasingly popular with Western consumers due to their minimal processing and alleged health benefits. Despite this trend, the management of vegetable fermentation still relies heavily on empirical knowledge, as the microbial communities and metabolic processes underlying the production of safe and nutritious products still need to be better understood. Indeed, the microbial community of most fermented vegetables is spontaneously established, and metataxonomic studies have shown that fermented vegetables may present different taxonomic profiles during and at the end of product fermentation. Shotgun metagenomic studies are powerful complementary methods to improve the characterization of a microbiome by (i) refining the taxonomic profile to identify key bacterial and fungal species and strains, (ii) providing insights about the metabolic potential of the community, and (iii) exploring the relationship/interactions between the diversity of taxonomic profiles and their functional profiles.

This study investigated whether shotgun metagenomic from various fermented vegetable samples with different microbial taxonomic profiles exhibit distinct functional potentials. We also explored the strain diversity among samples with similar microbial taxonomic profiles. To this end, we conducted a comprehensive analysis of the microbial diversity, taxonomic composition, and metabolic profiles of 141 samples. Among these, 124 samples were issued from nine independent public studies, and 17 were newly sequenced samples from the citizen science project "Flegme" carried out in France in 2022. Using a reproducible analytical workflow with a read-based approach (comprising the tools MetaPhIAn, HUMAnN, and StrainPhIAn), we were able to identify a core set of microbial species and strains and link them to a set of shared metabolic functions that could represent a network of activities important during the fermentation of vegetables. This study represents an original and extensive investigation of the microbiome of fermented vegetables, providing valuable insights into its composition and functionality.

S9-SF02 Metagenomic analyses revealed bacterial communities and diversities in fermented African locust beans

Afolake Olanbiwoninu, Yemi Fajire, Rachael Fashogbon Ajayi Crowther University, Oyo, Nigeria

Fermented African Locust bean (*Parkia biglobosa*) is one of the most widely consumed and important alkaline fermented condiments in many countries of West and Central Africa. It is rich in vitamins and used as a flavour enhancer in soup, and meat/protein substitutes. In order to



harness the beneficial importance, a deeper understanding of the composition and functionality of the bacterial species involved in the fermentation process is required, which was done using metagenomics. Ten (10) samples of the fermented African locust beans were collected from 3 towns in Oyo State, Nigeria. Illumina's next-generation sequencing was employed in studying the bacterial community and diversity of the fermenting African locust bean samples. A total of one hundred and forty-six (146) Amplicon Sequence Variants (ASVs) were obtained with varying taxons and confidence levels from the 10 fermented African locust bean samples. The sequences as revealed by the Phred quality score of the DNA bases from the bacterial species of the fermented African locust beans samples ranged from 27.5 to 38.5. The most predominant Phylum, Class and Order are Firmicutes, Bacilli and Bacillales respectively. The most frequently occurring genus in all the samples was Ureibacillus, while at the species level, Ureibacillus thermosphaericus had the highest percentage (77.09%). The lowest percentage was observed in *Chungangia koreensis* at 0.16%. The alpha diversity study showed that sample 9 had the highest microbial richness with a Chao1 index of 39 while the Shannon index showed that sample 10 has the highest diversity (2.800831). The principal coordinate analysis showed that samples 3, 4, and 8 were the most similar based on their microbial composition on the distance metrics. At the same time, there was a great differentiation in the microbial communities of samples 1 and 6 compared to the other samples. The 16S rRNA metagenomics identified a wide range of bacterial species associated with the fermented African locust beans, which could be used to determine that more microorganisms are involved in fermentation processes than the culture-dependent method.



Preservation of food systems microbiomes

S11-PL01 Underpinning the food systems microbiome through optimised preservation approaches

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In Europe, The EU Microbiome Support CSA project defined the microbiome as 'Microbiomes are complex communities of microorganisms that, together with their "theatre of activity", are characteristic of a particular habitat'. Further, they provide various crucial ecosystem services and are thus essential to the well-being of plants, animals and the environment and are therefore essential to the food system microbiome.

Culture Collections have a history of supporting microbiological research, primarily through the accession, preservation and supply of axenically cultured microorganisms. However, in nature microbes do not exist on their own, they interact with many millions of other microbes. With developments in technology, microbiome research is changing the way culture collections and biobanks need to support their user communities through the optimal preservation of samples and the curation of associated meta-data. Key requirements for biobanking have been proposed, including the development of standards, and has emphasised the need to deposit material and supply cultures, samples and associated data for future research. Additionally, this will provide a mechanism to protect intellectual property, and help researchers adhere to legislative and regulatory requirements, including the Nagoya Protocol of the CBD. Integral to the above is the need to preserve soil and plant samples and their microbiota.

Historically, culture collections have applied cryopreservation at ultra-low temperature and freezedrying protocols to preserve microbes. The microbiome, however, presents a greater challenge – how do we translate the methodology to complex samples that may contain many thousands of different species? The answer lies in our understanding of how microbial cells respond to the stresses encountered during freezing, thawing and recovery and how methods can be optimised to retain physiological and genomic integrity for different taxa and cell types. Using this approach, we can start to predict the components of the microbiome that may retain viability and, importantly, retain their functional potential. In this talk, an overview will be provided of how 'state-of-the-art' technologies are being developed, adapted, and applied to complex microbial samples and synthetic consortia through two ongoing projects: the EU Microbiome Biobanking (RI) Enabler and the UK Crop Microbiome Cryobank. The projects provide a blueprint for how biobanking, culture collection, and data networks can come together to support the needs of the academic and industrial research communities. Importantly, we believe that this model for biobanking of food and crop microbiomes can be translated to different systems across the world. Further, there is a responsibility on researchers themselves to observe good practice and deposit their strains in accessible, public collections to ensure the integrity and reproducibility of research outputs.

S11-PL02 The milk paradox

Christina Warinner

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Milk is a familiar food. And a fraught food. The natural food of mammalian infants, milk was first transformed into dairy products more than 9,000 years ago in the Near East, making it one the earliest human-manufactured foods. Remarkably, its production required the domestication of not only animals, but also that of bacteria and yeasts –



microorganisms too small for humans to see or even perceive. Dairy technologies spread alongside prehistoric migrations and social upheavals throughout Europe, Africa, and Asia over the next 5,000 years, were introduced to the Americas and Australia during colonialism, and then became a key vehicle of Cold War diplomacy during the 20th century. Today, dairy products are produced and consumed worldwide, and annual global milk and dairy production exceeds 700 million tons. And yet the majority of the world's population is estimated to be lactose intolerant. How did such an unlikely and often indigestible food become a staple of global cuisines? Building on emerging technologies in the archaeological and biomolecular sciences that are making dairy products visible in the archaeological record for the first time, this talk examines the long and often surprising history of milk. Using an interdisciplinary approach, I will take a fresh look at the phenomenon of lactose intolerance, its history of scientific study, and its unexpected ethnographic and archaeological paradoxes. Far from familiar, I aim to show that milk is revolutionary food with an ancient origin and a modern microbiome mystery at its heart.

S11-ST01 Holistic approach to study soil rhizosphere microbiomes and preservation strategies.

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Holistic approaches are required to better understand the distribution of microbial diversity and the functional profile of communities in soil microbiomes. The One Health approach by the World Health Organization considers soil as a pivotal factor for animal and human nutrition and its preservation as an important goal to achieve a higher and worldwide well-being of the planet.

In the present work we investigated how different sample preservation techniques can affect the soil rhizosphere microbial communities by analysing the culturable fraction of microorganisms, the microbiota and the metabolic fingerprint. Three different storage conditions were considered: at -80°C, lyophilization and refrigeration (4 °C). Microbial community structures and growth strategies were investigated by means of total microbial count and enumeration of distribution between copiotrophs and oligotrophs bacteria, r-k strategists and EPi index were calculated. A total of 300 bacterial strains, 54 putative N-fixing strains and 315 fungi were isolated for further investigation of plant growth promoting activity. Functional-level metabolic profiling of microbial communities based on Biolog EcoPlates highlighted widespread carbon sources utilisation in samples with high population diversity, confirmed by metagenomic analysis, such as kiwifruit and strawberry, when compared to poor species abundance soils, as grapevine.

Linking the distribution of microbial diversity and ecosystem functioning is essential to understand community responses to changing environment, especially in forced conditions such as samples preservation. For that reason, the same analysis will be performed on samples at 6 and 12 months and results will help better understand structural shifts during microbiome conservation and which functional features in the community can be affected during long term storage. In parallel, within isolates showing PGP traits, microbial consortia set-up will be carried out to exploit natural inhabitants of soil communities (NatComs) as biofertilizer to enhance crop productivity.

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Connectivity of microbiomes in the food system

S12-PL01 Microbiome interconnectedness throughout environments with major consequences for healthy people and a healthy planet

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Microbiomes have highly important roles for ecosystem functioning and carry out key functions that support planetary health, including nutrient cycling, climate regulation, and water filtration. Microbiomes are also intimately associated with complex multicellular organisms such as humans, other animals, plants, and insects and perform crucial roles for the health of their hosts. Although we are starting to understand that microbiomes in different systems are interconnected, there is still a poor understanding of microbiome transfer and connectivity. In this presentation we show how microbiomes are connected within and transferred between different habitats and discuss the functional consequences of these connections. Microbiome transfer occurs between and within abiotic (e.g., air, soil, and water) and biotic environments, and can either be mediated through different vectors (e.g., insects or food) or direct interactions. Such transfer processes may also include the transmission of pathogens or antibiotic resistance genes. However, here, we highlight the fact that microbiome transmission can have positive effects on planetary and human health, where transmitted microorganisms potentially providing novel functions may be important for the adaptation of ecosystems.

S12-PL02 Extensive microbiome mapping in the food industry as a strategy to identify functional landscapes for bioprotection and food waste reduction

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The microbiome occurring in the food industries and residing on surfaces and in ingredients has a significant impact on food quality and safety. The microbiome of food ingredients maybe integrated by environmental contamination from surfaces and tools and concur to determine the composition and functions of the food microbiome, with obvious consequences for its quality and safety. Microbes residing on surfaces in the food industries plants may contaminate intermediate products and actively participate in food fermentation, ripening or even spoilage depending on the characteristics of the specific surface microbiome. Within the MASTER EU project (Microbiome applications for Sustainable Food Systems through Technologies and Enterprise, www.master-h2020.eu) we performed massive microbiome mapping in the food industries, with most of the samplings made in cheese making facilities in at least 4 European countries. We sequenced and analysed metagenomes from food samples, raw materials and environmental swabs collected from both food contact and non-food contact surfaces. The plants were shown to harbour a very complex microbiome, which varied according to food type and specific facility. In the cheese industry, more than 1,200 samples were collected and analysed and the environmental microbiome was characterized by high prevalence of genes potentially involved in flavour development, probiotic activities and resistance to gastrointestinal transit, suggesting that these microbes may potentially be transferred to the human gut microbiome. Reconstruction of high-quality Metagenome Assembled Genomes (MAGs) allowed identification of genomes harbouring genes related to antibiotic resistance, highlighting how industry surfaces represent a potential hotspot for AR spreading along the food chain.

S12-ST01 Microbial communities of Taggiasca fermented olives and olive tree: preliminary study combining culture-dependent and independent approaches.

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The olive tree (Olea europaea L.) holds significant economic and agronomic value in the Mediterranean basin. In Liguria, North-West Italy, the Taggiasca olive cv. is cultivated in a landscape marked by steep hills and terraced slopes and is renowned for the production of extra-virgin olive oil and fermented olives. In this study, the microbial ecology of the spontaneously fermented fruits was analysed through culture-dependent and independent methods. Next, selected yeasts isolates were screened for their ability to withstand different salt concentration and incubation temperatures, as a preliminary screening for the selection of potential starter cultures. Finally, the understanding of the microbial olive tree ecosystem was carried out by analysing bacterial and fungal communities of below-ground compartments (rhizospheres and soils) with metabarcoding analysis. Taggiasca olives fermentation entailed the analysis of brines and olives at different time periods in two separate batches. Culturedependent analysis revealed that the most abundant species overall were Candida diddensiae, Wickerhamomyces anomalus, Pichia membranifaciens and Aureobasidium pullulans. Amplicon-based sequencing confirmed the presence and dynamics of W. anomalus in batch 1 in brines and olives throughout the fermentation, while Cyteromyces nyonsensis and Aureobasidium spp. dominated the fermentation of brines and olives in batch 2, respectively. For starters preliminary screening, 54 yeasts isolates were tested on YPDA supplemented with growing salt concentrations (0, 8, 10 and 12% w/v) and incubated at different temperatures (12, 25 and 30°C). While 32 out of the 54 isolates could grow at low temperatures in control media (0% NaCl at 12°C), only two Debaryomyces hansenii isolates showed visible growth after 24 hours under the most stressful conditions (12% w/v NaCl at 12°C), suggesting this specie is more adapted to highly salty environments. Further enzymatic tests are needed to understand to which extent they contribute to the fermentation process, while the microbiome of rhizosphere and soils in three different areas of the orchard will be determined according to an altitude gradient. This study represents a first effort towards the comprehensive analysis of the olive tree microbiomes and provides an insight into the development of starter cultures, that can improve the sensory quality and microbial stability of the final food product.



Closing Keynote

CL-PL01 Microbiomes at the crossroads: Pathways for sustainable food systems and the SDGs

Karel Callens

FAO, Italy

Global progress toward the Sustainable Development Goals (SDGs) is lagging, particularly in the context of agrifood systems, which play a critical role in achieving a broad range of these goals. Agrifood systems significantly influence major global challenges such as hunger,



malnutrition, health, climate change, and environmental sustainability but also hold the potential to drive transformative solutions. Agrifood systems have a dual role as both drivers of and solvers of these global challenges and are tightly interconnected with other critically important systems like health, social, energy, and water systems, among others.

Central to the discussion on agrifood systems transformation is the emerging science of microbiomes, which offers both profound new insights into the functioning of agrifood systems, and innovative pathways around some of the more devastating negative-sum trade-offs that have hampered transformative change towards sustainability and resilience. Microbiomes are pivotal across all aspects of the agrifood systems, including in enhancing soil health and productivity, increasing crop resilience, improving food safety, enhancing human and animal nutrition and health, reducing environmental and climate impacts, etc. By leveraging microbiome science and innovation, we can deepen our understanding of agrifood systems and introduce new pathways for innovative, sustainable practices that support the achievement of the SDGs.

There is an urgent need for a paradigm shift in how we perceive and manage agrifood and other interconnected systems, and the full-scale integration of microbiome science into our collective understanding, and in global frameworks and initiatives such as the One Health Initiative, Codex Alimentarius, the Intergovernmental Panel on Climate Change (IPCC), the Global Soil Partnership, etc. There is need for UN and other international organisations, like FAO, to foster more robust dialogue and functional interfaces across scientists, policymakers, and the public, alongside support to governments and their development partners for revisiting policies, regulations, and investments that will harness the transformative potential of microbiomes, thereby facilitating a more sustainable, resilient, and equitable global food system aligned with the Sustainable Development Goals.



Poster abstracts

Poster Session 1: Microbiomes and the fertilizer crisis

PS1-S1-PP01 Study of the application of bio-based materials for the delivery of plantgrowth promoting bacteria

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The strong dependency on chemical fertilizers in agriculture poses major issues in terms of energy consumption, CO_2 emissions, water eutrophication and soil degradation. Also, the amount of food wastes within the agri-food sector represents an evident ethical and environmental problem. In horticulture, the replacement of plastic plantlet containers with bio-based configurations derived from food wastes has the potential to increase the overall production sustainability in a circular economy perspective. These biodegradable nursery pots reduce transplant stress and labour and can be further functionalised by embedding microbial fertilizers, to increase plantlet growth and reduce the need of agrochemicals. The developed bio-based materials can be also exploited for the realization of powders, beads, or seed coating for the delivery of beneficial bacteria to crops.

The aim of this work is to study the delivery of plant growth promoting (PGP) bacteria within a biopolymer obtained from food wastes. Plant-associated bacterial strains isolated from different host plants were selected based on their *in vitro* PGP properties, abiotic stress tolerance and polysaccharidases activity. The ability of bacteria to promote plant growth was further assessed on several plant species under greenhouse conditions. On lettuce plants, *Rhizobium* sp. GR12 and *Bacillus* sp. LR01 showed positive effect on plant height, nitrogen-flavanol index and nitrate content in leaves.

Cell viability within different biopolymer prototypes was evaluated by embedding the strains into the liquid material and by isolating them after its solidification and drying, simulating a possible shelf-life. Spore-forming bacteria such as *Bacillus* sp. LR01, LR20 and RP26 demonstrated to be stably viable within a pectin and cellulose film up to one month after the biopolymer storage. The average living cell concentration spanned between 10^4 and 10^8 cfu/g of material. The strains were successfully released from the film when in contact with soil and were able to colonize the roots of lettuce plantlets. *Rhizobium* sp. GR12 was tested in sodium alginate beads and survived for two weeks at a concentration of 10^5 cells per dry bead. Further studies are ongoing using fluorescent engineered strains to evaluate their route from the delivery biomaterial up to the plant rhizosphere and endosphere.

PS1-S1-PP02 Reduced nitrogen fertilization enriches nitrogenfixing bacteria in the *Brassica napus* seed microbiome across successive generations

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Brassica napus L. (oilseed rape) is a significant global crop that undergoes



intensive breeding and cultivation impacting both the plant microbiome and the environment. Here, we analysed the impact of different nitrogen (N) fertilization regimes under large-scale field trials using amplicon sequencing, quantitative real-time PCR, microscopy, and germination assays. Two consecutive years of either low or high N fertilization resulted in a significant shift in seed microbiota of oilseed rape, with potentially N-fixing bacteria (Rhizobiales and Rhizobium) being significantly enriched after low N fertilization. Overall bacteria and bacterial nifH gene counts were significantly higher in seeds and roots under lower N availability. The alternation of N levels between the first and the second growing season (high to low, or low to high) had no impact on seed microbiota. In addition, high N levels correlated with increased seed-to-seed transmission, whereas low N levels correlated with an increased acquisition of environmental microbiota. In addition, we analysed the impacts of breeding lines, germination rate, pathogen resistance, and external factors on seed endophyte communities of ten different oilseed rape cultivars from 26 field sites across Europe. The cultivar was found to be the main driver of bacterial diversity, abundance, and composition, and specific bacterial biomarkers were identified to differentiate between host functional traits such as germination and resistance. Applying a Bayesian community approach suggested vertical transmission of seed endophytes, where the paternal parent plays a major role and might even determine the germination performance of the offspring. Our study suggests a flexible, soil-influenced seed microbiome assembly that underlines the potential of the microbiome to be implemented in crop breeding and biocontrol programs and that, especially in the long term, innovative microbiome management via biological N fixation have the potential to sustain mineral fertilizer applications in future agriculture.

PS1-S1-PP03 Effects of sustainable agricultural practices on soil microbial diversity, composition, and functions

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Soil microorganisms can provide multiple benefits to agroecosystems, and these benefits are assumed to be promoted by sustainable agricultural practices. This assumption is based on increases in microbial biomass that are indeed observed with the implementation of sustainable agricultural practices. Yet, the connection between these practices and the complex soil microbial composition, along with their related functions, remains unclear, despite their growing adoption. Accordingly, we searched field experiments worldwide contrasting soil microbial communities under conventional and sustainable agricultural practices. We analysed 924 results of relative abundance of bacteria or fungi (using 16S and ITS rRNA amplicon sequencing, respectively) at the Family taxonomic level obtained from 46 articles. We found higher soil bacterial richness and higher abundance of copiotrophic bacteria under sustainable agricultural practices. Organic fertilization promoted the abundance of bacteria involved in C and N cycling, while conservation tillage decreased those involved in plant decomposition. Sustainable agricultural practices had a minor effect on the overall fungal structure, yet they did increase symbiotic fungi. Also, we observed a slight increase in arbuscular mycorrhizal fungi and a slight reduction in pathogenic fungi associated with plant disease. Higher microbial taxonomic diversity did not lead to increased soil multifunctionality. However, it can indicate soil resilience, which warrants further study. This study establishes that sustainable agricultural practices can influence microbial communities, driving compositional changes and promoting specific functions. Altogether, it highlights the importance of integrating soil ecology into agricultural management for sustainable agriculture.

PS1-S1-PP04 Harnessing Nitrogen Fixation: Advances in Diazotrophic Bacteria Engineering for Sustainable Crop Production

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In recent decades, numerous studies have investigated the interactions



between diazotrophic bacteria and non-leguminous crop plants, aiming to find sustainable alternative to synthetic nitrogen fertilizers. However, the utilization of diazotrophic plant growth-promoting bacteria (PGPB) currently faces biological challenges that hinder their further advancement in sustainable agriculture. Primary among these challenges are the sensitivity of nitrogenase to oxygen (O₂) and the deactivation of nitrogenase activity by the ultimate product of nitrogen fixation, NH4⁺. Consequently, the potential for employing synthetic biology techniques to engineer diazotrophic bacteria in symbiotic relationships with plants is increasingly becoming conceivable.

To this end, spontaneous mutations were induced in two diazotrophs, *Azospirillum formosense* and *Stutzerimonas stutzeri*, using UV light. The aim was to obtain spontaneous mutants capable of nitrogen fixation in the presence of O_2 and NH_4^+ . To identify such mutants, UV-exposed cells underwent transformation with the broad-host range expression vector pTH1227 modified by incorporating the respective *nifH* gene of the two strains (with an additional 200 nucleotides upstream of the *nifH* region) upstream of the *gusA* reporter gene. Furthermore, the wild-type *A. formosense* underwent genetic manipulation, during which the ammonium-inducible gene dinitrogenase reductase ATP-ribosyltransferase (*draT*), a post-transcriptional deactivator of the nitrogenase enzyme, was effectively knocked out to maintain nitrogen fixation activity in the presence of NH_4^+ .

At present, no *gusA* activity has been observed in diazotroph UV mutants, further trials are ongoing. Nonetheless, the draT-deficient *A. formosense* strain exhibits altered cellular morphology, characterized by an elevated presence of vesicle-like structures. These structures are hypothesized to aid *Azospirillum* in protecting nitrogenase from O₂ exposure. Ongoing *in-planta* assays using the obtained mutants, aim to ascertain whether the UV-induced mutants or the *draT*-deficient strains can enhance plant growth rates compared to their respective wild types.

The successful engineering of diazotrophic bacteria with enhanced nitrogen fixation capabilities holds promise for revolutionizing sustainable agriculture practices by overcoming the natural limitations carried by known diazotrophs.

PS1-S1-PP05 Exploring the impact of intercropping camelina-pea on soil and rhizospheric microbiome dynamics and crop productivity

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Currently, intercropping systems (= simultaneously growing two crops together in the same piece of land) gained considerable attention from farmers and researchers due to their potential to enhance agricultural productivity and improve soil fertility via root exudates and key taxa. Legumes are frequently included into intercropping systems due to their capacity to fix nitrogen and their ability to facilitate the uptake of other nutrients. In this context, the present study aimed to compare camelina (Camelina sativa L.) intercropped with pea (Pisum arvense) vs. sole-camelina in terms of productivity, nutrient uptake, and soil microbial diversity. A field trial was established at the experimental organic farm of the University of Bologna in Ozzano dell'Emilia, adopting a randomized complete block design with four replicates. Shortly before crop harvest, from each plot soil and rhizosphere of 24 plants per treatment were sampled for DNA extraction. At harvest straw and seed yield were determined and then N and P uptake in each plant organ (straw and seed) were analysed. Results showed that intercrop-camelina produced greater straw yield than sole-camelina but no significant differences were surveyed for seed yield. In terms of N and P uptake, the statistical results mirrored straw and seed yield results: in the straw both nutrient uptakes were greater when camelina was intercropped compared to sole-camelina but no significant differences were found for seeds. Contrary as expected, the collected soil of intercropping system showed lower alpha-diversity compared to sole-camelina soil, while at rhizospheric level no significant variations were found. However, peas altered the rhizosphere population of camelina, leading to a significant increase of Gemmatimonadaceae, a slight increase of Solilubrobacteriales (0.05 < P < 0.1) and a significant decrease of Oxalobacteriaceae and Weeksellaceae. The present research demonstrated that camelina-pea intercropping caused significant shifts in nutrient uptake and bacterial diversity. The benefits, such as nutrient use efficiency, derived from intercropping should be closely tied to the alterations in soil microbial functions, and changes in crop communities could stimulate specific traits in soil microbial communities.

Poster Session 1: Microbiomes mitigating biotic and abiotic stress

PS1-S2-PP01 Bacterial plant probiotics modulate the endophytic community structure in grapevine micropropagated plants

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An improved understanding of bacterial colonization processes and outcomes could benefit the use of plant probiotics in the field, promoting the transition toward sustainable agricultural practices thanks to their ability to sustain the growth, health, and productivity of the holobiont.

In this study, we administered two beneficial bacterial strains, *Kosakonia* VR04 sp. and *Rhizobium* GR12 sp., to micropropagated grapevine cuttings obtained via somatic embryogenesis. Both strains colonized the plant endosphere, with different final outcomes on the plant and its endophytic community. In particular, *Rhizobium* GR12 sp. increased root biomass under a growth condition of nutritional deficit. Phylogenetic and co-occurrence analyses showed that the plant native microbiota, originally dominated by Streptococcaceae and Micrococcaceae, radically changed depending on the inoculation treatments. After 30 days of plantlets growth, *Pantoea* became a predominant taxon, and considering untreated plantlets as references, *Rhizobium* sp. GR12 showed a minor impact on the endophytic bacterial community, both regarding taxa relative abundance and microbe-microbe interactions. Indeed, *Rhizobium* sp. GR12 was able to preserve the native microbiome structure despite its effective colonization, highlighting the importance of the plant-endophyte associations for the holobiont performance. On the other hand, *Kosakonia* sp. VR04 determined a major change in community composition, suggesting an opportunistic colonization pattern. Coherently, no growth promotion effect could be detected in the plantlets inoculated with this strain.

Overall, the results of this study highlight the importance of preserving the native endophytic community structure and functions during plant microbiome engineering. Furthermore, we suggest the use of micropropagated plantlets as a valuable option to study the interplay among the plant, its native microbiota and the invader on a wider portfolio of species besides model plants, facilitating the application of new knowledge in agriculture.

PS1-S2-PP02 Evaluation of the association of a PGPB strain RCA25 with Introgression lines (ILs) of *Oryza rufipogon X Oryza sativa cv Vialone Nano*

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Rice (Oryza sativa) is a monocotyledonous plant and it represents the staple food for more than half of world population. Rice domestication and varietal selection in Europe (ssp. japonica) and in Asia (ssp. indica) improved desirable agronomic traits but also lead to lose different genetic traits, while the wild progenitor of cultivated rice, O. rufipogon, is considered a donor of genetic variability. Besides that, rice cultivation is facing different problems worldwide, including tolerance to **biotic and abiotic** stresses that cause loss of rice productivity, greenhouse gas emissions (CH4) and increase of heavy metals such as Arsenic and Cadmium with consequent impact on human health. The exploitation of the genetic variability of rice lost during domestication and a beneficial root-associated microbiome can sustain rice growth and nutrition also reinforcing its defence against pests. Analysis of various crops and their wild relatives highlighted differences in the composition of the root/rhizosphere microbiome (such as AMF and PGPB) and variability in genetic loci involved in establishing positive symbiosis in tomato and barley. The microbiota associated with wild relatives evolved under marginal soil conditions, thus representing an untapped resource for low-input agriculture. In particular, the wild rice O. rufipogon accession 602-131-2 showed an aptitude to associate with the nitrogen fixing endophytic strain RCA25 at least sixty times more than Vialone Nano (O. sativa ssp. japonica), a renowned variety currently cultivated in Italy, thus foreseeing a possible reduction of chemical fertilizers. Research activities carried out within the Micro4Life project are addressed to the identification of O. rufipogon loci involved in the association between rice roots and the PGPB strain RCA25 by using introgression lines (ILs) BC_3F_5 obtained from crossing Vialone Nano with O. rufipogon 602-131-2. These ILs are being screened to detect their capability to associate with RCA25, by applying a specific protocol for seedlings inoculation. Phenotyping for the association aptitude with the tested strain is being conducted by counting CFU obtained from roots homogenate. Preliminary data are showing an outstanding variability in the association with RCA25, thus reflecting a genetic variability which will be further characterized through transcriptomic analysis of ILs showing contrasting association aptitude. Research within the AGER Micro4Life project (Rif. 2022-2903).

PS1-S2-PP03 TRIBIOME project: The influence of abiotic stressors on the wheat microbiome

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The EU's TRIBIOME project aims to assess the sustainability of food processes and enhance productivity under climate change scenarios by characterizing human, soil, animal and plant microbiomes to balance food production and ecosystems preservation.

In this context we investigated soil and rhizosphere microbiome of soft (*Triticum aestivum ssp. vulgare*) and hard (*Triticum turgidum ssp. durum*) wheat under drought conditions. Samples were collected from separate soft and hard wheat fields in Italy and Spain, subjected to drought (treatment) or not (control), during a standard yearly production cycle. We focused on samples collected during the flowering phase (April 2023). A total of 272 samples, including 30 root and 4 soil samples for each condition, were processed through 16S rRNA gene metabarcoding sequencing for taxonomic and phylogenetic analysis.

Our preliminary results show significant differences in root and soil microbiomes irrespective of condition or species in both countries. The microbial community α -diversity was higher in all control samples compared to treatments. However, intraregional variations showed contrasting patterns between countries. Italian rhizosphere samples highlighted a higher microbial ecosystem diversity compared to the respective soils under both control and treatment conditions, while Spain displayed an opposite trend. Moreover β -diversity analysis excluded significant differences between the rhizosphere microbial community between all control and treated samples regardless of plant species. Spanish treatment samples showed a significant increase of the genera *Promicromonospora*, *Streptomyces* and *Variovorax* while respective Italian samples showed a significant increase of the genera *Microlunatus*, *Rubrobacter*, *Flavisolibacter*, *Pedobacter*, *Azospirillum* and *Microvirga*. Species within the last two genera were observed to promote crop growth, reduce nitrogen fertilizer requirements, establish beneficial relationships with roots and perform plant growth-promoting (PGP) functions, with some *Azospirillum* strains described for their ability to confer plant tolerance to environmental stresses like drought.

These preliminary results provide insight into shifts in the wheat microbiome in climate change dynamics, enabling knowledge on PGP microbiome activities as a useful biotechnological tool for the implementation of sustainability in food systems.
PS1-S2-PP04 Augmenting zinc phytoremediation via Miscanthus rhizobacteria: Microbial approaches to enhance plant functionality and alleviate abiotic stress

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The repeated application of chemical fertilizers over time has been found to increase the accumulation of heavy metals, including zinc (Zn), in agricultural soils. This, coupled with the climate change crisis, which exposes plants to high temperatures and water scarcity, present a significant obstacle to sustainable agriculture. In this work, we tried to address these major challenges using PGPR for enhancing Zn phytoremediation by miscanthus, a sustainable bioenergy crop plant.

A total of 70 rhizobacteria, obtained from miscanthus grown in Zn-spiked pot soil, were *in vitro* screened for PGP activities (phytohormone and siderophore production, nitrogen fixation, phosphorus solubilization), drought stress mitigation (growth under osmotic stress and ACC deaminase activity) and zinc bio-adsorption. Two strains were selected as plant inoculants also considering the possibility to exploit a microbial cooperation phenomenon known as "microbial hitchhiking" which could provide a synergistic effect between the strains in the consortium, potentially making the treatment more effective.

Evaluation of the PGPR inoculation on plant in drought and zinc stress was performed in a pot experiment by comparing the yield and status of plants grown in presence or not of PGPRs, under the diverse stresses. Moreover, the microbiome response to microbial inoculant was evaluated with 16S metabarcoding which allowed to compare the bacterial population of miscanthus roots in different conditions.

In summary, this research suggests that PGPRs represent a viable approach for remediating Zncontaminated soils with miscanthus plants during drought conditions. Priming the chosen strains to withstand heavy metal and drought stress, as well as identifying effective delivery methods for microorganisms to inhabit the plant rhizosphere, are crucial stages in formulating microbial-based bioaugmented phytoremediation techniques.

Poster Session 1: Animal welfare, antibiotic resistance, and robustness

PS1-S3-PP01 Metagenomic insight into microbiome and resistome of probiotics, starter cultures, and cheeses

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The presence of antibiotic resistance genes (ARGs) in the food microbiota is a concern for public health and food safety. Despite extensive research on resistomes in humans, animals and the environment from a One Health perspective, food systems remain largely unexplored.

Our aim was to analyse the microbiomes of cheeses, probiotics and starter cultures using shotgun metagenomic sequencing to gain new insights into their resistomes.

We isolated metagenomic DNA from 75 samples, comprising 12 probiotic food supplements, 13 starter cultures, 16 raw milk cheeses with starter cultures, 17 raw milk cheeses without starter cultures and 17 cheeses from thermally treated milk with starter cultures. Sequencing was carried out using the Illumina NovaSeq platform. Paired-end reads were de-multiplexed, trimmed, filtered, and assembled *de novo*. ARGs were identified by mining the coding sequences with Ublast. Sequencing and bioinformatic analyses were conducted partly by Microsynth AG (Balgach, Switzerland) and partly by the authors.

Taxonomic profiling revealed that bacterial units predominated across all samples, with *Bacillota* as the dominant microbiota, comprising 92.45 % of the total relative abundance. At the genus level, *Lactococcus* and *Streptococcus* were particularly abundant with a relative abundance of 37.77 % and 30.64 %, respectively. Analyses of α -diversity and β -diversity indicated that the microbiota of raw milk cheeses and probiotics exhibited the highest diversity, while starter cultures and cheeses produced from pasteurised milk with starter culture showed significantly lower diversity. Sequencing data revealed a total of 663 ARGs conferring resistance to various antimicrobials, including disinfectants (*clpL*), tetracyclines (*tetU*, *tet(M*)), aminoglycosides (*ant(6)-la*, *aph(3')-III*), beta-lactams (*PBPs*, *ampH blaACC-3*) and macrolides (*mdf(A)*, *lsa(A)*), which were also the most abundant. We demonstrated that raw milk cheeses had a significantly higher α -diversity and relative abundance of ARGs compared to probiotics, starter cultures and cheeses produced from thermally treated milk with starter cultures.

Our findings indicate that thermal treatment coupled with the simultaneous use of starter cultures leads to diminished microbiome and resistome diversity in these samples. Furthermore, starter cultures and probiotics do not serve as a substantial reservoir of resistance compared to raw milk cheeses.

PS1-S3-PP02 Detection, spread, and transmission of antimicrobial resistant bacteria from swine farms to the surrounding environment.

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Antimicrobial resistance is a major threat to global health. The pig food chain is considered an important source of antimicrobial resistance genes (ARGs) and of antibiotic resistant bacteria (ARB). However, knowledge of the spread of ARGs and ARB within this food chain is lacking, posing a serious threat not least to the slaughterhouse workers. In the present study, we longitudinally followed two swine farms in Italy from the weaning phase to the slaughterhouse. We comprehensively assessed the diversity of ARGs, their diffusion, and the ARB encoding for these ARGs. Seven different environments were sampled, namely the pig gut microbiome, the soil surrounding the farm, the air within the farm, the animal drinking water, the wastewater, the workers' boot soles, and the slaughterhouse. We obtained metagenomic sequencing data from 294 samples. We identified a total of 530 species-level genome bins (SGBs), which allowed us to assess the dispersion of microorganisms and their associated ARGs in the pig food chain. We identified 309 SGBs able to spread from the animals to the soil surrounding the farms through wastewater, manure, and the workers' boots. 11 SGBs were found in all seven environments, hence we refer to them as "cosmopolitan" SGBs.

The 309 SGBs were characterized by a diverse and complex resistome, with 176 ARGs active against 18 different classes of antibiotic compounds, well matching antibiotic use in the pig food chain in Europe. Specifically, resistance to nitroimidazole, multidrug, glycopeptide antibiotics, tetracycline, Fosfomycin, phenicol, antimicrobial peptide, bacitracin, elfamycin, beta-lactam, amynoglicoside:aminocumarin, diaminopyrimidine classes were found at prevalences above 60%. Some of these ARG are located on mobile genetic elements, underlining their possibility to be transferred horizontally. Considering all the ecosystems, we found 107 ARGs, 18 of which were present with different abundance in all the samples, proving their wide distribution. The presence of a complex and scattered resistome can have a high impact throughout the swine food-producing system.

Our results highlight the need and urgency to implement more effective countermeasures to limit the spread of ARGs from pig food systems. We also demonstrate the relevance of metagenomics-based approaches to monitor the spread of ARGs for the safety of the farm working environment and the surrounding ecosystems.

PS1-S3-PP03 EU-CIRCLES project: Evolution of pig microbiota and health under different farming conditions.

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This study aimed to track the evolution of the pig's microbiome from early colonization to slaughter, focusing on identifying microbial species and functions associated with the health status of pigs raised in different rearing conditions. Ninety-six piglets, from 22 litters, were divided into two groups — PC1 (Production chain 1) and PC2 (Production chain 2) — at 21 days of age and monitored until slaughter, pigs were fed the same diet through the whole trial. Faeces and blood were collected at day (d) 21 (before weaning, T1), d42 and d80 (weaning unit, T2 and T3), d98 and d278 (fattening unit, T4 and T5), with slaughter occurring at d297 (T6). Shotgun microbiome analysis, blood formula, oxidative stress and immunoglobulin levels were examined. Rearing condition significantly affected species-level microbiome and functional profile at T2, T3, T4, and T5. At T3, piglets in the PC1 had a lower species alpha diversity (P=0.002) and a higher abundance of Prevotella sp. P5-92 (LDA=4.0, P.adj<0.01), L. amylovorus (LDA=5.1, P.adj<0.01), and B. porcum (LDA=4.8, P.adj<0.01) compared to those in the PC2, which were characterized by L. jhonsoni (LDA=4.9, P.adj<0.01) and L. reuteri (LDA=4.7, P.adj<0.01). Additionally, at T3 microbial pathways associated with drug and xenobiotic metabolism were more active in pigs in PC1 (log2FC=1.5, p.adj=0.04) that also showed a higher blood Neutrophil/Lymphocyte ratio (P<0.001). These findings underscore the significant impact of farm-specific conditions on pig microbiome development and health status.

PS1-S3-PP04 Creation of an "Egg-to-Meat" biobank of microbiota collected from broiler chickens raised with or without outdoor access.

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Chicken is the most consumed meat worldwide, with a variety of farming schemes from intensive indoor to free-range systems which raises different concerns in terms of animal health and welfare. Recent studies suggest that microbial fluxes in chicken are influenced by the aviary environments, the diet, the broiler's genotype and that various and successive microbial contamination or dispersion events occur in the production chain. A better understanding of these fluxes could help to identify potential levers for controlling microbiota and guaranteeing the health and robustness of animals, as well the safety of the meat. as The objective of our project was to assess the effect of outdoor access of broiler chickens on bacterial fluxes along the production chain. Microbiota samples were collected from eggshells to carcasses as well as from aviary environment: 1) to analyse microbiota compositions using 16S rDNA amplicon sequencing and search impact of housing (indoors vs outdoor access) on chick colonisation, on the dynamics and shaping of the microbiota during breeding and on the composition of carcasses bacterial contamination; 2) to build a biobank of microbiota. Several zootechnical parameters have also been monitored.

This challenging and extensive study was made possible by the gathering of the varied expertise of numerous partners in a consortium involving two Poultry experimental units (EASM and PEAT, INRAE, France) and five joint research units (INRAE, France) conducting researches respectively on the biology of farmed birds (BOA), on Food Microbiology for Health (MICALIS), on Infectious Diseases (ISP), on the control of the microbial hazards throughout the food chain (SECALIM) and on applied mathematics and informatic (MAIAGE) as well as a microbial Biological Resource Center (mBRC) dedicated to animal and human health (CIRM-BP). A biobank of 620 samples constituted by 2 pools of eggshells, 366 droppings, 92 ceca, 100 carcasses and 60 environmental samples were dispatched in a total of 1981 aliquots preserved at -80°C. The sampling points encompassed eggshells just after hatching, chick droppings and environmental samples 2 and 15 days after hatching, just before outdoor access (day 28), at day 47 and 61 and finally at day 63, ceca and carcasses of individualized chickens at slaughterhouse.

The biobank of samples is now available for further studies allowing reanalysis of certain samples if necessary or new analyses such as a culturomics approach.

Poster Session 1: Food systems microbiomes and epidemics

PS1-S5-PP01 Eco-evolutionary factors supporting microbial persistence in food processing environments

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Foodborne pathogens, such as *Listeria monocytogenes*, can survive in food processing environments (FPEs) for long time periods, with the potential to contaminate food products. This persistence of food-associated pathogens poses public health and economic risks with far-reaching implications.



Despite research into genetic traits of persistent strains, the phenomenon of persistence remains poorly understood.

The goal of the FoodSafeR project is not only to understand the genetic factors underlying persistence, but also the ecological and evolutionary dynamics supporting *L. monocytogenes* survival in FPEs, as microorganisms do not exist in isolation in nature nor in the built environment. In our multi-facetted approach, we first performed a meta-analysis of whole genome sequencing studies toward the study of *L. monocytogenes* persistence to investigate clonal complex specific patterns and identify whether persistence can be linked to specific genes. In parallel, we investigated the prevalence and traits of persistent strains of *L. monocytogenes* across Austrian food producers. As these analyses revealed no clear pattern to adequately explain persistence, we additionally sampled FPEs for *L. monocytogenes*, co-occurring community composition, and environmental parameters.

We hypothesize that ecological factors and evolutionary dynamics may play a key role in patterns of persistence, including interactions with the environment and other microorganisms, as well as evolutionary dynamics such as pangenome openness, horizontal gene transfer, and mobile genetic elements. By drawing upon concepts from microbial ecology and evolution, we aim to elucidate factors supporting *L. monocytogenes* persistence in FPEs toward the development of more predictive and targeted strategies for risk reduction and elimination.

PS1-S5-PP02 Shotgun metagenomic investigations to detect zoonotic agents in the food system: A CIRCLES project showcase

Valentina Indio, **Yitagele Terefe Mekonnen**, Chiara Oliveri, Alessandro Seguino, Federica Savini, Andrea Serraino, Marco Candela, Alessandra De Cesare University of Bologna, Italy

The Regulation EC 178/2002 states that European Member States should adopt measures aimed at ensuring that systems exist to identify and respond to food safety problems. The identification of food safety problems in the food system is mostly based on food controls, by competent authorities and food business operators. While targeted controls in foods are certainly useful to monitor the foodborne biological hazards, we are already aware of, the monitoring of the food systems with untargeted methods allows to have a more representative picture of dynamics of biological hazards circulating in a food system which can change on time. In the European project CIRCLES, we investigated the microbiomes circulating in commercial poultry food systems. Comparing the microbiomes, as determined by shotgun metagenomic, of the chicken caeca with those on the corresponding carcasses in two broiler groups from two different poultry houses and reaching the slaughterhouse in two different days, a significantly higher abundance of Chlamydia was quantified on carcasses in comparison to caeca (P = 0.003). The Chlamydia reads identified on carcasses with a relative abundance ranging between 3.8 and 88.1% (mean= 59.8%) were mostly belonging to the species C. abortus (mean = 30.7%), C. psittaci (mean = 19.0%) and C. ibidis (mean = 3.9%). The transmission of *Chlamydia* from poultry to workers have been described in the literature, while the transmission from humans to carcasses has not been previously reported. The two hypothesis which might explain the observed relative abundance of Chlamydia reads on carcasses are the circulation of the pathogen in the slaughterhouse aerosol and/or the ability of *Chlamydia* to form biofilms in the slaughtering line, resulting in cross contamination of the poultry carcasses along the line, up to the end of the refrigeration tunnel. These results represent a showcase of how shotgun metagenomic investigations allow to identify reads of traditional and emerging zoonotic agents in the poultry food system promoting further targeted investigations allowing the early detection of food and human biological hazards before they become a risk for human health.

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PS1-S5-PP03 Fresh produce sampling method development for reducing host DNA

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Foodborne illness occurs from the consumption of food contaminated with microorganisms and is a global public health concern. Fresh leafy produce provides great nutritional and health benefits however it is a particular food commodity that has seen an increase in microbiological related food safety incidents due to their ready to eat nature. To keep our food safe, we need to control different variables throughout the food chain. Microorganisms live and work in a community with various members providing synergistic and mutualistic activities to the whole community. However, there is little known about the microbial community through the food chain with most of the focus on the detection of food borne pathogens at the retail stage. By utilising omic technologies like metagenomics we can gain an understanding of the microbial community present throughout the food chain and what functions they can provide which can lead to developing novel control methods reducing spoilage and foodborne outbreaks. A common issue in microbiome studies utilising metagenomic sequencing is how to deal with the host DNA. This in an important concern for the food microbiome as too much host DNA limits the depth and number of reads associated with the target community. For amplicon sequencing the use of peptide nucleic acid clamps can reduce the proportion of plastid and mitochondrial DNA which cross-amplify with the bacterial 16S gene however these are not applicable with shotgun metagenomic applications. As such alternative sampling methods are one option which may allow for the reduction of host DNA. Ten sampling methods, ranging from stomaching, blending, sonicating in two different diluents, or milling of fresh, liquid nitrogen or previously sonicated leaves, were selected from the literature. The sampling methods were assessed using RT-PCR and amplicon sequencing to compare copy number and alpha and beta diversity metrics. All alpha diversity metrics returned significant differences between sampling methods for both 16S and ITS samples. These significant differences in the sampled community were also reported in beta diversity analysis across PCoA, ANOSIM and PERMDISP. Stomaching with 0.1% Tween 80 was identified as a low host DNA sampling method for the food microbiome and will allow for a deeper understanding of the microbial community at any point along the food chain and ultimately improving food safety.

PS1-S5-PP04 Longitudinal analysis of microbial diversity and dynamics during storage of chicken products: Towards early warning of risks posed by foodborne pathogens.

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Culture-free, direct analysis of the food microbiome, could offer a more timely assessment of all relevant species present in a matrix than classical



microbiological methods. Furthermore, longitudinal analysis of food-related microbiomes allows for the detection of meaningful interactions between key microbial species, some of which can function as early warning biomarkers for microbial hazards and risks on food and in the food production chain.

We have studied the diversity and dynamics of bacterial food microbiota of twenty-four chicken products representing four levels of an animal welfare quality mark (zero to three stars). The microbiota of the chicken products were analysed at three different time points: day of purchase (t1), after three days (t2) and after seven days (t3) of storage at four degrees Celsius. In addition, we determined the presence of *Listeria monocytogenes*, a foodborne pathogen by using classical microbiological methods and whole genome sequencing of isolates. We analysed the data by using a suite of different clustering algorithms and machine learning techniques.

The general trend for all products was a drastic drop in the alpha diversity of the bacterial community between t1 and t2 followed by a moderate decrease or slight increase between t2 and t3. On average a higher alpha diversity was observed at t1 for the three star products compared to the one or zero star products, but this difference was no longer significant (t2) or present (t3) at the later timepoints. Spoilage organisms such as *Brochotrix, Carnobacterium, Leuconostoc, Serratia* and *Pseudomonas* were most often found to become the dominant genera after time, although we observed some distinct associations between the animal welfare quality mark and specific genera. The *Listeria* status (+/-) was also a distinctive factor in clustering patterns and showed significant associations with the quality mark. Finally, we observed several associations within and between genera/orders that potentially indicate synergistic and/or antagonistic interactions.

These results indicate the importance of monitoring the microbiome of food-related matrices and the potential to extract meaningful data from these analyses, ultimately contributing to the forecasting of microbial hazards in the food production chain.

PS1-S5-PP05 Antimicrobial and probiotic properties of potential bacteriocinogenic lactic acid bacteria from African traditional foods of animal and non-animal origins

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The increasing spread of antibiotic-resistant pathogens is a growing concern in the agrifood industry. The growing inefficiency of conventional antibiotics requires the use of safe and sustainable alternatives for the biocontrol of pathogens and microbiome modulation. To this end, we characterized the antimicrobial and probiotic potential of bacteriocinogenic lactic acid bacteria (LAB) from African traditional foods of animal and non-animal origins.

In this study, LAB were isolated from African traditional foods, including *Fura*, *Wara*, *Dambu*, *Burukutu*, *Ogiri*, *Kindirimo*, *Kunu*, *Nunu*, Iru, and Palm wine, and identified using API 50 CHL and 16S rRNA gene sequencing. The Cell-free supernatants (CFS) from the LAB were characterized for specific antimicrobial substances by neutralization of the pH with 1M NaOH, treatment with Catalase enzyme, and boiling at 100°C for 20 minutes, and then evaluated against foodborne and zoonotic pathogens using the agar-well diffusion method. LAB strains that produced antimicrobial substances against the pathogens were further evaluated for tolerance to simulated gastric juice (pH 2.0), bile salt, phenol, auto-and coaggregation, hydrophobicity, hydroxyl scavenging, and competitive exclusion of pathogens.

Out of the 689 LAB isolated from the African traditional foods of animal and non-animal origins, 26 produced heat-stable antimicrobial substances with inhibitory activity against *Escherichia coli* O157: H7, *Pasteuerella multocida, Listeria monocytogenes, Salmonella enterica, Enterococcus faecium, E. faecalis,* and *Staphylococcus aureus*. The 26 LAB strains showed varying degrees of significant survivability to simulated gastric juice, 0.4% bile salts and phenol, auto-and coaggregation, hydrophobicity, hydroxyl scavenging as well as competitively excluding some of the indicator pathogens with higher viable counts ranging between 2.4 to 5.8 log cfu/mL.

The findings from this study reveal notable antimicrobial and microbiome modulatory properties of LAB isolated from African traditional foods of animal and non-animal origins and could be effective for pathogen control and colonization resistance in food and animal production systems.

PS1-S5-PP06 Microbiome investigation in Austrian hard-cheese production reveals taxonomic and functional networks between surfaces and food products at different stages of production

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Austrian Vorarlberger Bergkäse (VB) is an artisanal raw milk brine-washed hard cheese manufactured in the western part of Austria (Vorarlberg) and is produced from alpine cows' raw milk without the addition of ripening cultures. VB and its associated microbiota have been studied in the last years and certain bacterial taxa, such as Brevibacterium, Corynebacterium and Staphylococcus species, have been found to be highly abundant on VB rinds throughout ripening and in the processing environment (Schornsteiner et al., 2014; Quijada et al., 2018,2020) and to have significant transcriptional activity towards the degradation of milk components and the generation of organoleptic compounds (Quijada et al., 2022). Within the frame of the MASTER EU Consortium (https://www.master-h2020.eu/) 13 VB dairies were selected for the sampling of raw materials, food products (fresh & ripened), and foodcontact (FCS) and non-food-contact (NFCS) surfaces in different rooms through production (i.e. processing, cold storage, ripening and packing) and subjected to shotgun sequencing (n=269). The microbiome analysis revealed a clear difference between pre-and post-ripening environments, both taxonomically and functionally. Some of the earlier described Brevibacterium, Corynebacterium, and Staphylococcus were dominant in ripened VB rinds, greatly influenced by the brine and the surfaces of the ripening room. Conversely, the ripened VB core remained similar to the fresh VB, dominated by the bacterial starters. The bacteria on the ripened rind were not starter-associates and thus transferred during the ripening process via various environmental surfaces such as FCS and NFCS in the ripening, processing, and packaging rooms. The data created a facility-specific transmission map, predicting the potential sources of microbes and functions. Also, we observed genes encoding key enzymes for cheese ripening, such as those related to the generation of compounds like diacetyl and 2,3butanediol, which are crucial for cheese's flavour and aroma. The methodology applied also allowed us not only to define the microbiome in VB production with a high level of detail but also to seek the occurrence of potential spoilers and harmful socies and functions (e.g. the generation of biogenic amines) and their particular location within a company, setting a toolbox that can be useful for stakeholders towards a more safety and hygienic cheese production and the improvement of their organoleptic properties.

PS1-S5-PP07 Microbial diversity in plant-based meat alternatives

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The market demand for alternative meat products, driven by a reduction in animal-based diets, has given rise to a new generation of plant-based meat alternatives (PBMAs). While there has been a consistent upward trend in sales and product diversity over recent years, the published literature in this area remains primarily focused on market research and food technology. As the popularity of PBMAs continues to grow, concerns have surfaced regarding the safety and nutritional implications of these innovative products. Despite the requirement for all examined products to undergo heating before consumption, consumers lack familiarity with this product category, emphasizing the need for further research into product safety.

To address these concerns, we conducted an analysis of 32 PBMAs available in Austrian supermarkets. Utilizing 16S rRNA gene amplicon sequencing, the investigation revealed that the majority of the products were predominantly populated by lactic acid bacteria (specifically Leuconostoc or Latilactobacillus) and exhibited generally low alpha diversity. We believe that the prevalence of heterofermentative lactic acid bacteria is particularly significant for product stability and quality, with the potential to enhance the shelf life of these products. The remaining portion of the products was dominated by members of the Pseudomonadota phylum, such as Pseudomonas and Shewanella. In addition to lactic acid bacteria, a wide variety of different Bacillus species were identified, along with some Enterobacteriaceae and potentially pathogenic strains through our culturing approach.

Moreover, we characterized over 400 isolates (selective and non-selective plating of fungi and bacteria), and found species with whole genome sequencing that can be linked to foodborne illnesses (for example, Bacillus_A paranthracis carrying non-hemolytic enterotoxin genes, Staphylococcus aureus carrying AMRGs, and adherent-invasive E. coli). The existence of these species within this relatively limited sample set suggests that this product category could potentially act as a vehicle for foodborne pathogens in cases where kitchen hygiene is inadequate or if the recommended heating procedures are either absent, inaccurately formulated, or disregarded.

PS1-S5-PP08 Microbiota dynamics and differences in growth patterns among *Listeria monocytogenes* and *Listeria innocua* strains in UHT (Ultra High-Temperature) and raw milk at 4^oC.

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The genus Listeria compromises 27 recognized species of ubiquitous small rod-shaped Gram-positive bacteria. Listeria monocytogenes is the causative foodborne pathogen of listeriosis, one of the most serious foodborne diseases due to its high hospitalizations and case fatality rates. Although Listeria innocua was initially considered a non-haemolytic and non-pathogenic Listeria species, rare cases of L. innocua septicemia and meningitis infections have been previously reported and natural atypical haemolytic isolates have been isolated from different food products. Milk samples can become contaminated by Listeria spp. through intramammary infection; from faecal or environmental contamination of the udder surface; and as the result of post-pasteurization contamination due to poor sanitation practices. As a result of L. monocytogenes contamination, dairy products have been associated with approximately half of the reported listeriosis outbreaks in Europe and the United States. Currently, no studies have evaluated changes in the microbiota of raw milk when Listeria spp. grow in dairy products. Firstly, L. monocytogenes F2365 (epidemic strain causing 142 cases of human listeriosis), and L. innocua growth was monitored in UHT (Ultra High-Temperature) milk and raw milk at 4 ºC. Mesophilic aerobic bacterial counts in raw milk at 4 ºC were also monitored. Four biological and three technical replicates per isolate and type of milk were used on each sampling day. Secondly, the Gompertz model was applied to estimate differences between the kinetic parameters. A metagenomic approach was used to study at day 14 (exponential phase) and day 28 (stationary phase) the microbial community of raw milk samples after the contamination with L. monocytogenes and L. innocua isolates. The V3 and V4 variable regions of the 16S ribosomal RNA gene (16S rRNA) were sequenced by Illumina Miseq 300×2 sequencing technology including 5 biological replicates/strain. Differential kinetic parameters of L. monocytogenes and L. innocua grown in UHT and raw milk at 4 °C were observed. The alterations in the microbiota of refrigerated raw milk after the contamination with both strains were also assessed. This study highlights the importance of understanding the behaviour of pathogenic and non-pathogenic Listeria strains in different milk environments, offering valuable insights into mitigating listeriosis risks in dairy products.

PS1-S5-PP09 Real-time volatilomics of food microbiome: The potential of providing temporal dimension in multi-omics studies.

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Fermentation stands as a prominent exemplar of bioprocesses driving sustainable innovation in food systems. It harnesses microbial diversity to



enhance the overall quality and safety of the final products with low energy/environmental impacts. The diverse fermentation processes also represent model ecosystems of interest for basic studies in microbiology and for the design of bio-tools inspired by nature. These trends justify the rising need to comprehensively understand and optimize fermentation processes exploiting the huge potential of 'omics' technologies. Metabolomics, one of 'omics' approaches, offers a comprehensive insight into the metabolic state of microorganisms and the dynamics of metabolic pathways throughout the process. Within this context, microbial Volatile Organic Compounds (mVOCs) are intriguing metabolites linked with microbial metabolism, affecting sensory perception and consumer acceptance of food. Untargeted profiling of VOCs generated or consumed during fermentation provides valuable metabolomic information about the studied microbial ecosystems, identifying markers to monitor fermentation dynamics and to develop solutions to optimize product yield, quality and safety. Proton Transfer Reaction coupled with Time-of-Flight Mass Spectrometer (PTR-ToF-MS) is an analytical tool belonging to Direct-Injection Mass Spectrometric (DIMS) technologies. PTR-ToF-MS, coupled with an autosampler and tailored data analysis, represents a non-destructive method that ensures real-time analysis, a high degree of automation, and heightened sensitivity in experiments. PTR-ToF-MS has demonstrated compelling capabilities for online evaluation of the microbial volatilome across diverse fermentative processes of agro-industrial significance. We report a selection of case studies to underline the potential of PTR-ToF-MS for volatilomics in food microbiology and microbiome studies, highlighting the potential of providing temporal dimension metabolomics approaches. This feature is of relevant prospective interest to integrate the temporal perspective in multi-omics studies, an aspect of particular interest for constantly evolving systems such as microbiomes of food interest. We developed this overview in the framework of the projects 'iNEST', 'ONFOODS' and 'SUS-MIRRI.IT' [Italian National Recovery and Resilience Plan (NRRP) projects financed by the European Commission's Next Generation EU programme].

Poster Session 2: Microbiomes for improving health and well-being

PS2-S8-PP01 Probiotic bacteria *Lactiplantibacillus plantarum* is detected by primary neural cortical cells inducing transcriptional, morphological and functional changes.

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The interaction of bacteria with various somatic cell types is an exciting emerging field. Despite the known effects of microbiota on the gut-brain axis, very little is known about the direct interactions that bacteria could have with neurons, both in terms of molecular mechanisms and information transfer. In order to study these communication mechanisms, this study designs an in vitro model to co-culture microbiota-bacteria Lactiplantibacillus plantarum with neural cortical cells and analyses the effects of this process in both populations. Here, we show how bacteria and neurons can be cocultured, and demonstrate a novel integrated platform that facilitates the analysis of neuronalbacteria communication. The results we obtained showed that L. plantarum is capable of adhering to the surface of the neural culture and the amount of attached bacteria increases with co-culture time. In addition, neural co-cultured cells undergo changes in gene expression patterns and induce morphological and functional changes in the expression of key proteins in neuroplasticity such as Synapsin I and pCREB. Finally, using real-time optical (calcium signalling) readouts, we show that neural cells react to the presence and co-culture with bacteria cells increasing cytoplasmatic Ca²⁺ signalling. Our proof-of-principle data reveal crosstalk between these co-cultured cells and illustrate a novel example of cross-kingdom communication between highly diverse cell types. The ability to eavesdrop on information passing between these two very different levels of biological organization will facilitate insight into evolutionary cell biology and could impact the understanding of brain-bacteria communication for diagnosis or treatment of neuronal states in health and disease.

PS2-S8-PP02 Determination of some *in vitro* probiotic properties of bacteriocinogenic *Enterococcus faecium* H108 and H206 strains

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Enterococci can be found in many environments including food, soil, human and animal gut microbiota. Although they are not included in GRAS (Generally Recognized as Safe) status, some enterococci strains are used as starter, adjunct and probiotic cultures. Their bacteriocin production ability and resistance to harsh environmental conditions make enterococci attractive for use as starter, adjunct and probiotic cultures. The aim of this study was to investigate some *in vitro* probiotic properties of bacteriocinogenic *Enterococcus faecium* H108 and H206 isolates from raw milk. As a result of *in vitro* probiotic tests, H108 and H206 strains showed 89.32% and 93.37% resistance to simulated gastric juice for 3 hours, respectively. In addition, both strains grew in medium containing 0.3, 0.5 and 1% bile salts. *E. faecium* strain H206 had very high hydrophobicity with xylene, toluene and n-hexane hydrocarbons. In addition, both strains had bile salt hydrolase activity and produced hydrogen peroxide. On the other hand, the strains were found to contain at least one of the *gelE*, *efaAfs* and *esp* genes but none of the *cylA*, *hyl*, *asa* and *ace* genes. In conclusion, *Enterococcus faecium* H108 and H206 strains have the potential to be used as probiotic cultures in foods, but these properties should be revealed by more detailed *in vivo* and *in situ* studies.

PS2-S8-PP03 Probiotics and gut microbiota-brain axis: Exploring bioelectrical communication through enterococci

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Emerging evidence underscores the pivotal role of the microbiota-gut-brain (MGB) axis in the etiology and progression of certain neuropsychiatric and neurological conditions, attributed to dysbiosis and alterations in bacteria-neuron signalling. Notably, inter-bacterial communication via bioelectrical signals within biofilms suggests potential implications for long-distance signalling within the MGB axis. Despite the recognized effects of enterococci, inherent members of the human microbiota, as probiotic adjuncts in addressing intestinal dysbiosis, their bioelectrical properties and interactions with neurons remain largely unexplored. Here, we investigate the bioelectrical profile (electroma) of the ubiquitous Enterococcus faecalis (E. faecalis) and its responsiveness to neural stimuli, aiming to start elucidating the interplay between nervous signals, microbiota, and probiotics. Using a voltagesensitive fluorescent dye, bis-(1,3-dibutylbarbituric acid) trimethine oxonol (DiBAC4(3)), as a membrane potential (Vmem) indicator, we revealed and quantified i) the dynamic evolution of the bioelectrical profile during *E. faecalis* growth, ii) its response to two types of neural signals – the inhibitory neurotransmitter γ -aminobutyric acid (GABA) and the excitatory neurotransmitter Glutamate (Glu), and iii) the consequent impact of neurotransmitter-induced bioelectrical changes on bacterial growth, viability, and culturability, evaluated through absorbance readings, live/dead fluorescent probes, and viable counts. We observed a substantial impact of growth dynamics and neurotransmitters on the bioelectrical profile of bacteria. Notably, there was a marked increase in Vmem levels (depolarization) throughout bacterial growth. However, both types of neural signals significantly decreased membrane depolarization, while leaving growth, viability, and culturability unaffected. These findings deepen our comprehension of E. faecalis' involvement in gut-brain communication and provide insights into the effects of enterococci-based probiotics on the gut microbiota-brain axis.

PS2-S8-PP04 E. coli and the microbiota-gut-brain axis: Bioelectrical connection

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The complex interaction between the gut microbiota and the central nervous system, known as the microbiota-gut-brain (MGB) axis, has generated significant interest in recent years, leading to the emergence of neuromicrobiology as an exciting interdisciplinary field. Among the millions of resident bacteria, Escherichia coli (E. coli) is emerging as a promising probiotic platform for developing strains with the potential to regulate various metabolic and multifactorial diseases. Exploring the communication pathways between E. coli populations and neurons could unveil novel nutritional approaches to target the MGB axis. Here, we track and functionally alter the bioelectrical profile (electroma) within an *E. coli* population using the voltage-sensitive fluorescent dye DiBAC4(3) and the administration of established neurotransmitter drugs. Our goal is to elucidate the evolution of the bioelectrical profile throughout E. coli growth and its response to the inhibitory neurotransmitter yaminobutyric acid (GABA) and the excitatory neurotransmitter Glutamate (Glu). Furthermore, we assess the impact of neurotransmitter-induced bioelectrical changes on bacterial growth, viability, and cultivability through absorbance measurements, live/dead fluorescent probes, and viable counts. Our results demonstrate significant alterations in the bioelectrical profile of *E. coli* depending on the growth phase and neurotransmitter exposure, characterized by a decrease in depolarization in both scenarios. Interestingly, neurotransmitters do not affect bacterial viability or cultivability, suggesting that changes in membrane potential are solely attributed to neural stimuli and not to fundamental shifts in bacterial physiology. This investigation expands our comprehension of *E. coli*'s bioelectric behaviour within the gut microbiota and highlights the potential for external stimuli to influence bacterial bioelectrical signalling, offering implications for strategies targeting the MGB axis.

PS2-S8-PP05 *In vitro* assessment of the effect of commercial botanical products on the human faecal bacterial community structure

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The surging popularity of botanical extracts in dietary supplements has



prompted a closer examination of their potential impact on the gut microbiota. Despite historical research predominantly focusing on prebiotic activity, these extracts harbour compounds with broad-spectrum antimicrobial activity. This study aims to assess the impact of three commercially available plant-based food supplement ingredients (Bromeyal®, Axtragyl®, and Kalita® derived from pineapple, *Astragalus membranaceus* roots, and bergamot, respectively), owned by Giellepi SpA, on the human gut microbiome through an *in vitro* faecal incubation model and on single selected bacterial strains.

Testing involved three concentrations of the three products post *in vitro* digestion, adhering to the INFOGEST protocol, or in their original form. A microbiologically stable faecal suspension, emulating an active microbiota, was prepared using glycerol-supplemented faecal samples and stored at -80°C. Assessments included faecal suspension incubations in anaerobic conditions with the products. Metataxonomic analysis by 16S rRNA gene amplicon profiling enabled comprehension of bacterial diversity and abundance in the samples. Cultivation of selected individual bacterial strains (e.g., *Bifidobacterium* spp., *Bacteroides fragilis*, and *Odoribacter splanchnicus*) in the presence of the products was also performed.

The three products exhibited no adverse impact on the faecal suspension microbiota, maintaining stability in α -diversity, β -diversity, and most major bacterial taxa during incubations, while some species, genera, and bacterial families displayed moderate alterations. The cultivation of most single strains in the presence of the products revealed no significant differences in viable counts compared to the control, even at concentrations surpassing those realistically achievable through product consumption.

This study pioneers the assessment of gut microbiome harmlessness of specific commercial botanical products. Findings demonstrate minimal influence on the faecal microbiota structure, with partial inhibition of groups linked to potential negative health impacts and partial promotion of health-enhancing bacteria.

In conclusion, the tested food supplements ingredients, despite containing molecules with documented antimicrobial activity, can showcase a positive impact on health without disrupting the equilibrium of the human intestinal microbiota, rendering them microbiome-friendly.

PS2-S8-PP06 *In situ* phytate degradation by *Bifidobacterium* spp. as a novel approach to tackle micronutrients deficiencies in early life.

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Phytic acid is the main form of phosphorous storage in plant seeds. Due to its polyanionic nature, phytic acid chelates mineral cations such as iron, zinc, calcium and magnesium, both in food matrices and in the stomach, resulting in insoluble phytate salts. Monogastric animals, like humans, lack sufficient intestinal phytase enzymes to break down phytates, which pass undigested through the gastrointestinal tract, impeding the absorption minerals complexed to it. In low- and middle-income countries (LMICs), where complementary foods are mainly plant-based, such as cereal or pulse porridges, phytate significantly contributes to micronutrient deficiencies, especially in weaning children.

Phytase encoding genes have been identified in a variety of microorganisms (e.g, *Aspergillus niger, E. coli*), however only a few phytase degrading strains have been identified among *Bifidobacterium* spp., with *B. longum* subsp. *infantis* ATCC15697 being among the most active *Bifidobacterium* described. Being coloniser of the infant gut, Bifidobacteria could find valuable application as probiotics for early life nutrition. Sufficient phytase activity in the gut could allow for *in situ* phytate degradation and offer an innovative approach to improve mineral absorption from plant-foods.

In this study, a collection of 50 *Bifidobacterium* spp. strains (species *B. longum*, *B. catenulatum*, *B. pseudocatenulatum*, *B. breve*, *B. bifidum*, *B. animalis*) was evaluated for phytase activity *in vitro*. Phytase producing strains were identified among members of the *B. longum* subsp. *longum*, *B. longum* subsp. *infantis* and *B. catenulatum* species, exhibiting comparable phytase activity to the reference strain *B. longum* subsp. *infantis* ATCC15697. The strains were sequenced and annotated, allowing bioinformatic identification of candidate phytase genes. Genes encoding candidate phytases were cloned and heterologously expressed in the host *B. breve* UCC2003, which lack phytase activity, and the resulting recombinant strains were assessed for their phytase activity. Genes encoding phytases were identified in *B. longum* subsp. *longum* NCIMB 8809, *B. longum* subsp. *infantis* MB0047, and *B. catenulatum* subsp. *kashiwanohense* APCKJ1 and are being characterised. These findings deepen our understanding of phytase distribution and activity in the *Bifidobacterium* genus, laying the groundwork for tailored solutions to enhance micronutrient absorption from phytate-rich foods.

Poster Session 1: Microbiome diversity and food quality

PS1-S6-PP01 Exploring the diversity of bacterial communities in tempeh and its implications for food safety in Indonesia

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Fermented foods are attracting attention due to their potential health benefits. Research has demonstrated that the consumption of fermented foods can enhance the diversity of gut microbiota, which is linked to a healthy gut microbiome. The microorganisms present in fermented foods are believed to have positive effects on health. Tempe, a popular fermented food in Southeast Asia, lacks a comprehensive understanding of its bacterial community composition, function, and influencing factors. Using high-throughput sequencing, we conducted a thorough analysis of the microbial composition, diversity, and functional potential of tempeh collected from different locations in Indonesia. Our findings revealed that the hygiene practices during tempeh production significantly influence the microbial community composition and overall microbial function. Surprisingly, the packaging of tempeh also had a moderate impact on bacterial diversity. Employing a genome-centric approach, we discovered that bacteria associated with tempeh have the potential to provide beneficial functions, such as the production of vitamin B, vitamin K, and short-chain fatty acids. This study lays the groundwork for enhancing the quality of tempeh by modifying the indigenous microbiota, which is often overlooked.

PS1-S6-PP02 Exploring microbial changes during green coffee fermentation in apple juice and their influence on coffee aroma

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The consumption of coffee and coffee-based beverages is widely prevalent worldwide. Commonly, coffee cherries are fermented to obtain



green coffee beans which, once dried, are shipped in the different countries where they are roasted, processed and sold. Nowadays to obtain blending with new aroma characterizations, the coffee industries are starting to try a re-fermentation by using different liquid matrices as source of precursors for the correct microbial development.

In this study, the microbiota dynamics and the volatile component of a spontaneous green coffee fermentation were compared with one co-inoculated with *Pichia kluyveri* and *Lactiplantibacillus plantarum*. A commercial apple juice was chosen as the liquid medium for fermentation to impart specific flavour characteristics to the final coffee sensory profile.

Samples were collected at intervals of 24, 30, 48, and 72 hours and analysed for the microbial load and pH trend. The acidification rate of the two fermentations among the time followed the same trend. The results showed for lactic acid bacteria (LAB) a load after 72 hours, reaches around 9 Log CFU/gr for both spontaneous and co-inoculated fermentations although after 24 hours LAB load was significant lower for the spontaneous fermentation. GC-MS analyses for the volatile composition and shotgun metagenomic sequencing showed differences in the microbial function and development.

These findings provide valuable insights into a potential new coffee fermentation technique, offering promising avenues for innovation in the food industry.

PS1-S6-PP03 Is it possible to replace the traditional dynamic wild fermentation with preselected cultures?

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Nowadays, wild fermentation is still used by numerous producers, especially by traditional fermented vegetables producers. Traditionally kimchi, sauerkraut, fermented cucumbers etc. are produced with spontaneous fermentation, although it is a well-known fact that it does not always provide stable quality and sometimes even ends up with unsuccessful batch. Therefore, producers are interested in using preselected starter cultures, but there are two major obstacles to overcome: starter cultures must provide at least same quality sensory profile for the products and price of starter cultures should be acceptable.

The objective of this study was to investigate the dynamic fermentation process of kimchi and sauerkraut. In both cases wild fermentation was used, and the size of each batch was 100 kg. To understand the effect of environment fermentation was conducted in two environments: production facility and laboratory.

Analysis was conducted at the following time points of fermentation: 0-, 4th, 7th, and 14th day. HPLC was used to measure the chemical profiles (sugars, organic acids, and ethanol). For microbiological analysis, we used modified 16S rRNA sequencing technology that is able to discriminate between total and viable bacteria and provide the estimated number of cells. Descriptive sensory analysis was carried out in the sensory lab with trained assessors.

Bacterial composition results showed rather similar dynamic in kimchi and sauerkraut – in the earlier fermentation stage, the main bacteria belonged to the *Leuconostoc* genus, while in the later stage, the predominant bacteria were from the *Lactiplantibacillus* and *Levilactobacillus* genera. Similar conclusions can be drawn with sensory and chemical analysis results. The results also showed that microbiological consortia were similar in samples from both environments meaning that the impact from the environment was less significant.

The dynamical changes in different time points showed that the composition of starter cultures have to be relatively complex. It would be important to find minimal composition of starter cultures necessary to complete the fermentation at desired quality level. Therefore, different studies for wild fermentation are needed to understand the differentiation between producers, products, and environments. This will allow to create preselected starter culture that gives high stable quality products with possibly acceptable production cost addition.

PS1-S6-PP04 Application of transcriptionally active in-house microbiota on Austrian smear cheese: an early look

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The targeted extraction and use of the cheese dairy's own smear cultures has not yet been described in popular or scientific terms. The exact knowledge about the mechanisms that the individual bacteria and fungi on the cheese rind use to form specific metabolites is also limited.

The aim of this work is therefore to isolate in-house rind microbes from cheese factories, to determine them using whole-genome sequencing, to select suitable candidates and to use them for the targeted smearing of hard cheeses.

The hypotheses are that common microbes contain potential genes for the production of aroma compounds. And that the targeted selection and practical application when smearing the cheese with these microbes leads to a positive ripening effect on the cheese. This includes the development of a stable, healthy rind, freedom from undesirable rind defects and a more rapid development of the desired sensory properties in the cheese dough.

This project can therefore help ensure that fewer cheeses have to be discarded or withdrawn from the market due to defective rinds or undesirable sensory deviations. Furthermore, an intact rind with a healthy rind-microbiome reduces the risk of colonization with pathogens. A shorter ripening time until the typical smell and taste is achieved means more efficiency in production for the dairies. This saves costs and resources and enables cheese dairies to plan their processes better. Furthermore, the use of in-house microbes offers the advantage that even regional specialty producers with protected origins have the opportunity to adapt their product, where the purchase of smearing cultures is not permitted. From a scientific perspective, the precise determination of ripening microbes based on their genetic resources is a milestone in determining their potential for protection against pathogens, formation of aromatic substances, desirable (vitamins) or undesirable (histamine, biogenic amines) metabolites, and their ability to degrade proteins and fats.

PS1-S6-PP05 Changes in grape-associated microbiome as a consequence of chitosan pre-harvest application

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The microbiome associated with ripening grapes has been showed to be profoundly linked with the viticultural terroir and to play a key role in the winemaking process, determining the quality of final product; this



microbiome encompasses beneficial, innocent and spoilage microbial species. Among them, the fungus *Botrytis cinerea* causes grey mold of grapes, one of the most economically important diseases of grapevine worldwide. Sour or acidic rot, a further disease often associated with grey mold, is also caused by microorganisms, noteworthy microbial consortia containing acetic acid bacteria.

Chitosan is a naturally polysaccharide derived from chitin with a demonstrated potential to control several plant diseases and to extend fruits and vegetables storage life. Several reports have shown its antimicrobial activity, which can interfere with the growth of phytopathogenic fungi, including *Botrytis* spp. Furthermore, chitosan has been reported to activate plant defences and to enhance plant resistance to fungal infection.

A specific chitosan product derived from *Aspergillus niger* (LalVigne[™] Botryless, LB) was sprayed on Pinot Grigio grapes during the ripening (BBCH85) to control *Botrytis* development and sour rot subsequent appearance in a vineyard located in north-eastern Italy.

Aims of the study were to (a) evaluate the efficacy of LB-chitosan in controlling spoilage microorganisms, as *Botrytis*, acetic bacteria and other sour-rot associated taxa (b) investigate the impact of LB-chitosan on taxa other than its aforesaid direct targets, in particular on the beneficial microorganisms potentially involved in wine fermentation.

Samples of grapes were collected few days before harvest and two different conditions were analysed (with and without LB-chitosan application) in triplicate. To map the microbiome, Next-Generation Sequencing (NGS) was employed: fungal and bacterial communities were characterized through metabarcoding (ITS,16s) before and after the treatment.

A significant difference in abundance of genera as *Botrytis* (ITS) and Acetobacteraceae (16S) between treated and untreated samples was confirmed in the dataset. Moreover, differences in the microbial biodiversity were found between grapes from the two conditions. Interestingly, a variable evolution of microorganisms during the entire trial (4 days) was also found.

Overall, NGS metabarcoding proved to be an effective technique to study microbial communities when chitosan is applied before harvest.

PS1-S6-PP06 Characterization of microbial diversity in ready-toeat salad based on MALDI-TOF MS analysis and full-length 16S rRNA gene sequencing during cold storage

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The consumption of ready-to-eat (RTE) foods as a convenience and fresh meal with few preservatives has been increasing in recent years. The nutritional and economic values are impacted by microbial spoilage. This



study aimed to investigate the effects of different storage conditions on the shelf-life quality of MAP (Modified atmosphere packaging) mayonnaise-based potato salad. We characterized the microbial community using full-length 16S rRNA gene sequencing and compared it with classical microbiological methods paired with MALDI-TOF MS identification. Industrially produced salads were stored at varying temperatures (0, 4, 8, and 12°C) for 7 and 14 days. The total bacterial numbers, lactic acid bacteria numbers, and the numbers of Enterobacteriaceae were determined by plating according to the ISO standards, and the representative isolates identified by MALDI-TOF MS analysis. Changes in pH, TTA, and sensory profiles were analysed. The results showed that during the first week of storage, aerobic bacteria and lactic acid bacteria counts increased significantly at all storage temperatures 10²-10³ times depending on temperatures (P<0.05) Identification of bacterial population with MALDI-TOF showed that the dominating bacterial population after 7 days of storage at 0 and 4°C mainly consisted of Lactobacillus-related genera similar to the initial composition of salad, while a major fraction of Carnobacterium spp., Leuconostoc spp., and Hafnia was dominated at 8 and 12°C at the end of shelf life. The 16S rRNA microbiome analysis revealed that the most abundant phyla was Firmicutes and the four highly abundant species assigned to Latilactobacillus sakei, Leuconostoc inhae, Dellaglioa algid, Leuconostoc carnosum in the bacterial communities. The effect of temperatures and storage time was generally significant on both pH and TTA changes ($p \le 0.05$). After the first week of storage, the pH decreased to 4.2 in the samples stored at 8 and 12 °C. However, after 14 days, only samples stored at 0 °C had a pH higher than 5.8. The sensory profile remained relatively stable until 7 days of storage at low temperatures, whereas the perception of the overall intensity of aroma and vinegar attribute increased significantly at the end of storage ($p \le 0.05$). The combined culture-dependent method and 16S rRNA gene sequencing provide complementary results and give a better resolution of microbial community structure in a complex food to ensure food safety and quality.

PS1-S6-PP07 An investigation on probiotic properties and safety evaluation of non-starter lactic acid bacteria from traditional Turkish yoghurt

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Yoghurt, produced by *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*, is one of important fermented products worldwide. This product is produced industrially on a large-scale, but it is



still produced using traditional methods in many countries. Non-starter lactic acid bacteria other than the yoghurt bacteria may be present in traditional yoghurts due to the insufficient aseptic conditions in the homemade production. Therefore, it is quite important to determine beneficial and/or harmful effects on health of the non-starter lactic acid bacteria in yoghurt. The aim of this study was to investigate probiotic (tolerance to simulated gastric juice and bile salts, antimicrobial activity, auto/coaggregation, hydrophobicity, bile salt hydrolase activity, in vitro cholesterol lowering potential) and safety properties (antibiotic resistant profile, presence of virulence genes, etc.) of non-starter lactic acid bacteria from traditional yoghurt samples, including Enterococcus faecium YH36, Lactococcus lactis YH56, Limosilactobacillus fermentum YH35, YH42, and YH43. All isolates tolerated to simulated gastric juice by 37.88-91.53%, while to 0.3, 0.5, and 1% bile salts by 0.54-64.48%. E. faecium YH36, L. fermentum YH35, YH42, and YH43 strains inhibited two or more pathogens including E. coli O157:H7, S. aureus ATCC 29213, P. aeruginosa ATCC 9027, S. Typhimurium RSHMB 95091. All strains had autoaggregation (3.96-22.04%) and hydrophobicity ability (7.33-64.82%). Moreover, the isolates aggregated with E. coli O157:H7, S. aureus ATCC 29213, S. Typhimurium RSHMB 95091, and L. monocytogenes ATCC 7644 by 5.53-22.28%. E. faecium YH36 and L. lactis YH56 lowered cholesterol level by 71.57-4.14%, respectively. All isolates were sensitive to ampicillin, erythromycin, clindamycin (except E. faecium YH36), tetracycline, chloramphenicol, while resistant to vancomycin (except E. faecium YH36 and L. lactis YH56), gentamicin (except L. lactis YH56), kanamycin (except L. lactis YH56), and streptomycin (except L. lactis YH56). In addition, gelE and efaAfs virulence genes were detected in L. lactis YH56 and L. fermentum YH42, respectively. These results showed that there could present potential probiotic non-starter lactic acid bacteria with some virulence genes in traditional yoghurts. Finally, it was revealed that not only yoghurt bacteria but also non-starter lactic acid bacteria should be investigated in terms of food safety and human health.

PS1-S6-PP08 Delving into the potential of new *Lachancea thermotolerans* strains to improve the organoleptic profile of Pale Ale beer

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The beer industry has recently shown a special interest to increase the available commercial offer by focusing on the study of alternative yeasts, among others. Non-*Saccharomyces* are noteworthy due to the importance they are achieving and their important impact in final product. The majority of non-*Saccharomyces* species used in brewing belong to *Pichia kluyveri*, *P. anomala*, *P. kudriavzevii*, *Lachancea thermotolerans, Candida stellata, C. krusei* and *Rhodotorula mucilaginosa*. However, few of them have been employed in the industrial preparation of beer, either alone or in combination with *Saccharomyces*.

For this purpose, the brewing potential of yeasts collection belonging to different species of non-*Saccharomyces* isolated from southern Spain was evaluated. Thus, all the yeasts were characterized in terms of fermentative vigour, sugar assimilation, off-flavour, and hop resistance. Among all of them, different *Lachancea thermotolerans (Lt)* strains showed the most promising brewing profile because of their high resistance to alpha acids, no production of off-flavour compounds and they were able to finish the fermentation without the presence of *Saccharomyces* yeasts. Furthermore, *Lt* strains displayed a high L-lactic acid production above 2 g/L.

Hence, to verify the effectiveness of these selected *Lt* strains, Pale Ale beer style was elaborated using the following simple inoculations: *Saccharomyces cerevisiae* plus *Lactiplantibacillus plantarum* (control), *Lt* reference strain (Hansen), *Lt* BMA228, *Lt* BMA298, and *Lt* BMA339. All *Lt* strains could ferment the wort by themselves, without *S. cerevisiae* co-inoculation. Moreover, a volatile compounds production and sensorial analysis of the elaborated beers was carried out, being BMA339 which obtained the best overall scores and VOC performance.

Among all studied non-*Saccharomyces* strains, several *L. thermotolerans* strains displayed a great performance and therefore a great potential in sour brewing. However, additional tests must be carried out before their use at industrial scale.

Acknowledgements

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PS1-S6-PP09 Harnessing the microbiome for innovations in cocoa

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The cocoa industry faces systemic issues in it's the value chain. We're now in the third consecutive crop deficit due to poor harvests. Farmers deal with new plant diseases, dwindling arable land, and climate change disruptions. Further, the widely used spontaneous fermentation process yields beans of inconsistent quality, failing to meet quality standards which leads to wastage. The cocoa supply chain also faces ethical dilemmas, including human rights abuses, low pay, and child labour. Unregulated terms like 'fair trade', and 'organic' are used to meet consumers' demand but actual change has been limited. This research proposal hopes to address the quality issue by fermentation. The aim is to use microbes isolated from spontaneous fermentations of cocoa from central Africa and use them as an element of innovation in boosting chocolate quality. Rooted in academia, the research has the potential to address industry challenges from a systems perspective. Starter cultures, commonly employed in fermented foods like cheese and wine, have seen limited utilization in cocoa. This stems from insufficient characterization of microbial diversity in key cocoaproducing regions. To bridge this gap, my research employs next-generation technologies to delve into the microbial functions and interactions shaping cocoa fermentation processes, using culture dependant as well as culture independent methods. The research would thus advance our knowledge about the cocoa microbiome and the interactions within the ecosystem. The research has the potential to reduce wasted cocoa by introducing a starter culture during primary fermentation or post fermentation. By using a secondary fermentation starter, companies could revitalize low quality cocoa. The cocoa industry's complex challenges demand a systemic approach for meaningful change. This research, focusing on cocoa fermentation, aims to enhance chocolate quality and reduce waste, easing pressure on processors and infrastructure development in the rainforests to promote ethical sourcing. Additionally, exploring microbial genetic data opens the door for cellular agriculture already being explored by companies around the globe. Together, these efforts offer a pathway to transform the cocoa supply chain, benefiting farmers, consumers, and the environment.

Poster Session 2: Microbiomes for plant fermentation

PS2-S9-PP01 The bacterial microbiota of table olives: A metastudy

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Table olives are among the most ancient fermented foods and their production is strongly intertwined with the culture of Mediterranean countries. Three main trade preparations exist (alkali treated olives; naturally fermented olives; olives darkened by oxidation) but combinations of alkali treatment, drying, cracking, addition of spices result in a large variety of products, with different ecological conditions. The microbial ecology of table olives has been extensively studies, first by cultivation-based approaches and, recently, by metataxonomic and multiomic approaches. Results are dispersed in a large number of studies and, even if for several of these raw sequences are available in repositories, direct, quantitative comparison of results is hampered by lack of a coherent metadata structure.

By building upon FoodMicrobionet (https://github.com/ep142/FoodMicrobionet), we developed, in collaboration with CSIC, Seville, Spain, a set of controlled terms for manually annotating the samples, including information on cultivar, trade preparation style, etc., and developed a custom script for analysis of the results.

The current data set for bacteria, includes data from 10 studies published between 2013 and 2023, and 425 samples of olives, brines and food contact surfaces or material, for 5 countries and 8 olive varieties (both Greek-style and Spanish-style), throughout the fermentation processes.

The alpha diversity varies widely between and among samples, with Chao1 usually decreasing as the fermentation progresses. After taxonomic agglomeration and filtering, 158 taxa were retained out of the original 509. Several genera were found in both alkali treated and naturally fermented olives. The most prevalent and abundant genera include both *Pseudomonadota* (*Celerinatantimonas, Idiomarina, Marinobacter, Halomonas, Pseudomonas, Salinicola, Enterobacter, Acinetobacter*) and *Bacillota* (*Lactiplantibacillus, Pediococcus, Leuconostoc, Lentilactobacillus*). In most, but not all cases, bacterial microbiota of brines and fruits differed within the same variety. The microbiota of olives from different varieties differed more than those of Greek- or Spanish- style olives within the same variety.

The database size will progressively increase in size and variety during the METAOlive project, funded by MUR, Rome and EU, and we are confident it will become a valuable resource for scientists working on olives and vegetables fermentations.

PS2-S9-PP02 Lactic acid bacteria ecology changes along the Spanish style cv. Chalkidiki green table olives spontaneously fermented under high and low NaCl conditions using classical microbiological and molecular techniques

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Table olives are among the most recognizable fermented vegetables in the Mediterranean area. Currently, scientific efforts are directed to



position them as a functional food. Green table olives from cv. Chalkidiki, beside their economic impact, are appreciated in the Greek and international markets for their quality attributes. This preparation involves debittering with lye treatment (NaOH 2% w/v) and repeated washes followed by spontaneous fermentation in high NaCl concentration brine. Lactic acid bacteria (LAB) contribute substantially to table olive fermentation. However, the impact of debittering and brine salt content on LAB ecology of olive fermentation is not well understood. This study aimed to isolate and identify the dominant cultivable LAB at two different ecosystems at critical points of processing stages in industrial scale: firstly, the surface of olives (after harvest, debittering and at the end of fermentation as final products); secondly, the fermentation brine at the early and late stage of fermentation under high (8% w/v) and 50% partial replacement of NaCl with alternative salts. Following classical microbiological techniques (Gram staining, microscopic observation, catalase test), the bacterial isolates were subjected to PCR analysis. A total of 48 isolates were screened for LAB through the amplification of a hypervariable region of the 16S rRNA gene. Multiplex PCR analysis of the recA gene was applied to generate DNA fingerprint patterns of the LAB isolates and differentiate them between the species Lactiplantibacillus plantarum, L. pentosus and L. paraplantarum. The identities of the fragments were confirmed by sequencing. Evidence-based relations between LAB ecology and different ecosystems/treatments are thoroughly discussed.

Funding

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PS2-S9-PP03 Food matrix and disease prevention: *L. plantarum* fermentation as a tool to modulate bioactivity, digestibility and acceptability of pulse seeds

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The rise of noncommunicable diseases, driven by unhealthy diets, necessitates a reconsideration of food consumption as a preventive measure against diseases. Pulses, being rich in nutrients, fibers, and bioactive compounds, offer an ideal protein source for healthier and more sustainable diets. However, their consumption is hindered in Western countries due to low digestibility, off-flavours, and gastrointestinal discomfort.

In this study, we explored the potential of *L. plantarum* fermentation (0 to 72 hours) to enhance acceptability, digestibility, and bioactivity of pulses. The fermentation process significantly affected the seed-microstructure and led to de-glycosylation of phenolic compounds. We further examined peptides profile upon fermentation, *in-vitro* gastrointestinal digestion (INFOGEST), and transport across a Caco-2 cell monolayer by LC/MS/MS.

Fermentation of lentilf for 48-72 hours resulted in the formation of hydrophobic peptides and improved intestinal absorption. Moreover, exposure to fermented lentils led to over 30% increase in DPP-IV inhibitory activity in Caco-2 cells compared to unfermented lentils. Additionally, moderate fermentation of green lentils for 24-48 hours decreased the formation of flatulence-inducing raffinose-family oligosaccharides and improved the aroma profile by reducing grassy, beany off-flavours and forming pleasant aroma compounds.

Through the *ex-vivo* NuGUT continuous fermentation system, we observed that colonic fermentation of green lentils for 3 days increased the levels of beneficial commensal bacteria such as *Lactobacillus* spp. and *Roseburia* spp., which were retained when lentils were pre-fermented with *L. plantarum*.

Overall, our findings provide evidence that *L. plantarum* fermentation can effectively enhance the acceptability, digestibility, and bioactivity of pulses, encouraging their inclusion in daily diets.

PS2-S9-PP04 New fermented food product with enhanced bioactivity to minimize green pea (*Pisum sativum* L.) byproducts

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Green peas have a high waste index yielding a considerable amount of byproducts rich in bioactive molecules, particularly phenolic compounds that are recognized for their benefits to human health, including the reduction in coronary heart disease risk factors, prevention of several types of cancers, and modulation of immune and inflammatory responses. However, their clinical potential need still to be completely explored, and it can be potentially influenced by factors such as bioaccessibility, based on absorption and colonic fermentation, distribution, and metabolism. The structural complexity of polyphenols affects their absorption in the small intestine, with absorption rates often below 10%. Lactic acid bacteria (LAB) could play a crucial role in converting the glycosylated polyphenols into lower-molecular weight phenolic compounds such as phenolic acid thereby enhancing their bioavailability. Metabolites resulting from the degradation of plant polyphenols are believed to be responsible for the health-beneficial effects on the host. To address the challenges of valorisation of low-value food byproducts, this study aimed to develop a novel fermented sustainable food product with bioactive phenolic compounds derived from green pea byproducts. Initially, the effect of different LAB strains on a standard solution of different phenolic compounds added to MRS broth was assessed. Based on the obtained results, selected LAB strains were chosen to further ferment the pea byproduct puree. Phenolic compounds levels were determined using high-performance liquid chromatography with diode-array detection (HPLC-DAD) before and after the fermentation and the determined phenolic compounds were additionally confirmed by HPLC-QqQ (triple quadrupole mass spectrometer). Preliminary results showed that two strains of L. plantarum degraded protocatechuic acid, p-coumaric acid, kaempferol glucoside and methyl gallate suggesting tannase, decarboxylase and β -glucosidase activity in the MRS broth. Moreover, our results might suggest a similar enzymatic activity of selected L. plantarum strains in fermented green peas byproduct purees. Future steps involve conducting comprehensive chemical analysis to elucidate changes in the metabolomics profile at different times during the fermentation, in order to understand the modification of bioactive compounds and identify potential biomarkers for monitoring fermentation in relation to the sensory qualities of the final product.

Poster Session 2: The edible microbiome

PS2-S7-PP01 Ferments of the Future: A public-private partnership to accelerate research and innovation on ferments, fermented foods and biopreservation



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With the increase in environmental and climatic risks, society's expectations in terms of food have gradually changed towards a demand for safer, healthier and more sustainable food. Fermentation can be used to transform raw materials while adding new properties: taste, colour, texture, preservation, nutrients up to targeted health benefits. It was in that context that Ferments of the Future (FdF) was launched in late 2022, with €48.3m in funding from France 2030 over a 10-year period [1]. Its funding will provide unique, interconnected capabilities combining the very best in fermentation research and innovation. Focused on food, FdF will gradually expand into other sectors such as agriculture, health, feed and the environment.

To make ambitions a reality, FdF has brought together public-private ecosystem, currently comprising 42 members from higher education & research organisation and companies in the field of ferments, fermented foods and biopreservation.

Twenty people are currently working in this vibrant ecosystem, spread over several sites and managed by FdF:

- A Ferments du Futur Innovation Centre or "CI2F", located in the Paris-Saclay cluster and equipped with microorganism screening and characterization capabilities, multiple laboratory and pilot-scale liquid and solid fermenters, a state-of-the-art physico-chemical characterization platform and a food prototyping workshop.

- A "Distributed Platform" infrastructure, whose facilities reinforce existing scientific and technical platforms within seven INRAE research units (MaIAGE, MGP, Micalis, SayFood, SPO, STLO, UMRF) specialising in microbiology, process engineering, nutrition-health and applied mathematics-computing.

This one-of-a-kind infrastructure is open to collaboration with international research labs and European companies.

Acknowledgements

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[1] https://www.fermentsdufutur.eu/en/home/

Poster Session 2: Circular food systems for microbiomes improving animal, human and environmental health

PS2-S10-PP01 Searching for novel polyunsaturated fatty acid producers in terrestrial ecosystems implicated in rare-lipid provisioning and ecological services

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In-land production of long-chain polyunsaturated fatty acids (PUFAs) is thought to derive from eukaryotic microorganisms such as the thraustochytrid algal protists that inhabit freshwater ecosystems. In aquatic environments generally, a linear relationship is seen between PUFA content and organism taxonomic hierarchies - from algae to invertebrates to vertebrates. The scenario by which strictly terrestrial organisms obtain PUFAs such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) is incompletely understood. Curiously, soil bacteria such as members of the y-Proteobacteria and certain myxobacteria, such as Sorangium cellulosum and Aetherobacter fasciculatus contain genetic and enzymatic competences to denovo synthesize PUFA using an iterative type I fatty acid synthase (FAS)/polyketide synthase (PKS)-like synthase system. Additional candidate producers such as members of Planctomycetes or Chloroflexota are coming to light as metagenomic data unearths genome features of uncultured soil microorganisms. The gap between microbial and faunal food webs is linked by invertebrate macrofauna. Soil invertebrates such as nematodes, earthworms, and collembola can contain PUFAs between 10-30% of total fatty acids, and represent an overlooked source of omega-6 and omega-3 PUFAs. Expanding on these observations, we present evidence that terrestrial bacteria are part of the primary producer network for long-chain PUFAs, which macrofauna aggregate into their body tissue. We have interrogated metagenomic datasets, wholegenome archives, and Shewanella isolates from earthworm gut-soil to explore the connection between terrestrial microbial PUFA synthesis and ecosystem services that give rise to a major supply link in the animal food web. These data provide insights about how the terrestrial trophic chain may be supplied by bacterial PUFA synthase activity at its base, and explores the hypothesis that PUFA synthesis confers utilitarian membrane fluidic properties harnessed by deep-sea and terrestrial organisms alike. In a terrestrial context, fluidic microbial membranes may advantage redox cycling, lending detoxification services to the environment and simultaneously supplying a reservoir of nutritionally essential fatty acids. Understanding the extent and stimulus of producer networks is important for answering open questions in mammalian evolution, as well as for biotechnological efforts towards bioremediation and to sustainably source therapeutic PUFAs.

PS2-S10-PP02 Effect of probiotic bacterial supplementation on growth performance and bioconversion ability of *Hermetia illucens*

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Gut microorganisms play a pivotal role in the control of the overall wellbeing of their host. This is the reason why, lately, the attention has been



focused on the possibility of supplementing bacterial probiotics to the most economically relevant mass-reared insect species as the Black Soldier Fly (BSF) Hermetia illucens. BSF larva (BSFL) is indeed known for its stunning ability to feed on a vast amount of organic substrates, converting them into valuable larval biomass in compliance with the principles of circular economy. This work was performed to test BSFL ability to grow on different organic substrates and to gain insights into which bacterial species can produce beneficial effects on the host and can be considered promising candidates for future applications as probiotics. BSFL growth was first evaluated on different locally available by-products, i.e. okara, brewer's spent grains, potato peels and potato selection waste. BSFL was able to grow on all of them but with different efficiency degrees: the fastest development was observed on okara; on potato selection waste and brewer's spent grain the larval growth was slower but allowed anyway their bioconversion, while the poorest performance was observed on potato peels. Data were thus related to the ones obtained from the characterization of the gut bacterial community of BSFL reared on these substrates by 16S rRNA amplicon sequencing. We then decided to exclude the best and worst feeding substrates to further investigate the effect of bacterial administrations, in both active and heat-inactivated states. Specific microorganisms were isolated from the gut of H. illucens larvae grown on a nutritionally complete diet and selected because deemed capable of exerting a positive effect on the insect growth. Considering the obtained results, the supplement of active bacteria suggested a possible positive effect in a few cases, while heatinactivated bacteria administration seemed to be related to a higher final weight of the larvae and a slower prepupal development. In conclusion, further studies are needed to deepen our knowledge of the complex mechanisms underpinning insect-microbe interactions to be able to use them in future microbiome-engineering strategies.

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PS2-S10-PP03 Microorganisms for food tracking: the SeaTraceOmics project

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Seafood traceability is often challenging, especially when the species is the same, but geographical provenance makes the difference. However, environmental features can shape the microbial community, paving the



way for the use of a "microbial fingerprint" to obtain and predict information about an ecosystem. Thinking outside the box, such a microbial signature can be exploited for seafood traceability purposes.

Our case study focuses on the giant red shrimp *Aristaeomorpha foliacea*, which has a wide geographical distribution worldwide and represents an important economic resource, highly exploited in the Mediterranean Sea. However, new fishing grounds recently entered the global seafood market and began exporting their catch worldwide. This led to a differentiation in value of the species based on origin, making it susceptible to commercial fraud.

Thanks to broad engagement from several stakeholders, we were able to collect georeferenced samples from two relevant fishing areas. These areas are characterized by both economic and environmental diversity: the Strait of Sicily, where the Mazara del Vallo red shrimps are caught, and the Mozambique Channel.

Here, we implemented phase 1 of the SeaTraceOmics project with more than 800 samples analysed, comparing gut and gill microbiomes from the two different fishing areas. Our approach allowed for the taxonomic characterization of distinct bacterial communities linked to different ecological features, enabling differentiation of the two populations.

This study links microbial ecology to traceability and is at the core of the SeaTraceOmics project, constituting a promising avenue for investigating the ecological footprints influencing the sustainability and safety of seafood on our plates.

PS2-S10-PP04 Dynamics of the microbiota of brewers' spent grain during inoculated and spontaneous solid-state fermentation.

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Brewers' spent grain (BSG) is a cost-effective nitrogen-rich by-product of the brewing industry that is also highly susceptible to microbial spoilage.



Thus, different methods for the stabilization and valorisation of BSG have been implemented. In this respect, solid state fermentation (SSF) of BSG could represent a sustainable technology to promote the development of beneficial soil microorganisms and produce an N-rich soil amendment. The successful application of bioinoculants in non-sterile substrates (such as raw BSG or even soil) requires a thorough understanding of the relationships between exogenous microbial starters and the native microbiota of the substrate. This is crucial to prevent unexpected outcomes, such as the inability of the bioinoculant to thrive in the environment or a negative impact on the natural microbial ecosystems. Here, the microbial dynamics of freshly collected BSG subjected to an SSF process with a multi-kingdom microbial inoculant were monitored for 90 days at the laboratory scale. A spontaneous SSF of BSG was also carried out to assess the interactions between the bioinoculant and the resident microbiota. High-throughput sequencing of 16S and ITS rRNA highlighted the occurrence of three phases in the evolution of the microbiota during the SSF. Initially, the bioinoculant strongly affected sample microbial taxonomic composition, however starting from day 14, an increase in the number of resident microbial taxa was observed. Eventually, the bacterial and fungal genera included in the bioinoculant were unable to persist in BSG. Notwithstanding, the bioinoculant exerted a significant effect on the chemical and microbiological properties of BSG after 90 days. Cross-kingdom cooccurrence network analysis allowed to evaluate the interactions between the fungal and bacterial communities in shaping the microbial communities of the BSG. According to this analysis, the microbiota of inoculated BSG samples developed a stable network characterized by stronger and more significant correlations. The perturbance in the assemblage of microbial communities caused by the bioinoculant was also highlighted by the identification of specific keystone taxa. In conclusion, the interaction between the bioinoculant and the resident microbiota of agro-industrial wastes could result in completely unexpected outcomes, due to the nature of the inoculant, the process, and the waste microbiota.

PS2-S10-PP01 MICROBIOMES4SOY: Healthier diets and sustainable food/feed systems through employing microbiomes for soya production and further use

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MICROBIOMES4SOY addresses sustainable farming, climate change mitigation, sustainable aquaculture and the transformation of food



systems for health, sustainability and inclusion. The project addresses soya bean production and further use of soya bean as food and feed product. Microbiomes are explored along the food chain, from farm to fork to the animal and human gut with the aim to better deploy microbiota along the production chain to improve the quality and safety of food products. Furthermore, the aim is to better understand biological and ecological processes involved in the assembly and dynamics of microbial communities to be better able to develop microbiome-based application strategies. An important component of MICROBIOMES4SOY will be the strengthening of the relationships between all actors in our food system and to seek international cooperation, all along with early and wide communication of microbiome science and applications. A Stakeholder Board comprising key stakeholders in the farming, industry and policy sector will be involved in important communication and dissemination activities and will be invited to relevant workshops of the project to involve stakeholders in a participatory approach.

Poster Session 2: Preservation of food systems microbiomes

PS2-S11-PP01 Catabolic footprint of the microbiome of Sardinian sourdough by OmniLog[®] technology

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Sardinian sourdoughs, deeply rooted in the island's culinary tradition, harbour unique microbiomes that contribute to flavour profiles, textural and functional properties of the bread. Understanding the taxonomic composition and metabolic activities of these microbial consortia is essential for the preservation of the microbial biodiversity and the quality of Sardinian bakery products. In this study, OmniLog[®] PM technology (BIOLOG) was



utilized to characterize the physiological response of three previously taxonomically characterized microbiomes associated with 3 Sardinian artisanal sourdoughs (named 81, 82, 84). Particularly, over one hundred carbon (PM1 Plate) and nitrogen (PM3B Plate) sources were tested simultaneously. Microbial cells were isolated from the sourdoughs following the Standard Operating Procedures (SOPs) developed within the SUS-MIRRI.IT project, funded by the National Recovery and Resilience Plan-NRP ("NextGenerationEU" program). All the tested microbiomes were capable of catabolizing simple sugars (D-Fructose, D-Glucose, Sucrose, D-Mannose, D-Xylose, D-Ribose, D-Galactose, and L-Lyxose). However, there were differences in their overall catabolic footprint, with the microbiomes from sourdoughs 81 and 84 characterized by greater phenotypic diversity than that observed in sourdough 82, with 43% and 38% of fermentable carbohydrates catabolized, respectively. The microbiome from sourdough 81 was also capable of utilizing 88,54% of the nitrogen substrates, while microbiomes from samples 84 and 82 only 33,4% and 29% respectively. Nitrogenous sources used by all microbiomes corresponded to L-Methionine, L-Serine, L-Tryptophan, Ala-Gly, L-Norvaline, Met-Ala, Uric Acid, Ala-Leu, Gly-Asn, L-Glutamine, Ala-Gln, L-Ornithine, Gly-Gln, Gly-Met, L-Arginine, L-Aspartic Acid. Nitrogenous molecules, by interacting with sugars in the dough, take part in Maillard reactions and are responsible for different aromas and flavours in the crust. Additionally, the amino acids arginine and ornithine are the precursors of a wide range of compounds responsible for the aromatic and gustatory properties of the bread.

PS2-S11-PP02 Validation of standard operating procedures for DNA extraction and microbiome analyses of soil samples

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The development of Standard Operating Procedures (SOPs) for sampling, extraction and analyses of soil microbiomes represents a fundamental step in ensuring the reproducibility of results associated with the characterization and use of microbial communities. In order to validate previously developed SOPs, rhizosphere and bulk soil were collected from strawberry plants of cultivars Clery and Elodie in an experimental field in Boves (north-western Italy). Samples were homogenized and aliquots were sent to six Italian partners (UNITO, ENEA-CASACCIA, UNIPG, UNIVR, UNIMIB and UNIPA) to perform DNA extraction and quality assessment. Each group extracted DNA with the same commercial kit and following the same protocol. Characterization of fungal and bacterial communities was performed by means of metabarcoding in the same sequencing center, while data analysis was performed by each partner. All groups performed a successful extraction from all samples, although statistical analyses showed differences in the characteristics of the genomic DNA extracted by each group, both in quality (absorbance 260/280 and 260/230 ratios) and in quantity (ng/mL). Sequencing compounded to this imbalance, with UNITO obtaining a higher number of reads and, subsequently, a higher number of features compared to the other working groups. Compositional analyses of bacterial communities indicate the presence of an overwhelming effect of the teams involved in the test on the total variance (91%), with a minimal contribution from both matrix (4%) and cultivar (less than 0.1%), with significant differences between all working groups. For fungi, the "operator effect" exerted a much lower influence (38%) and comparable to the influence of matrix (39%), whereas the influence of cultivar remained very limited (4%); however significant differences among the working groups were highlighted. At the same time, analyses of the presence and relative abundance of fungal and bacterial taxa in the samples revealed a stable pattern in the identity of recovered taxa, with differences exclusively associated to their relative abundance. The study revealed that DNA extraction represents a key step in soil microbiome studies, independent from sampling and DNA sequencing, underlining the necessity to standardize the operating procedures associated with the extraction of DNA, such as the shipping time and conditions, the homogenization of the matrix, and the storage of the sample.

PS2-S11-PP03 Metabolic framework of spontaneous and synthetic sourdough metacommunities to reveal microbial players responsible for resilience and performance

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In the realm of microbial ecology, natural microbial communities are subject to dynamic shifts in composition, which can pose challenges to their resilience. In this study, we explore sourdough-a naturally occurring cereal-fermenting metacommunity—as a dynamic ecosystem influenced by continuous environmental and spatiotemporal stimuli. Through examination of spontaneous sourdough metagenomes and transcriptomes, we identified dominant, subdominant, and satellite microbial players engaged in various functional pathways. Microbial richness was positively correlated with the abundance of gene copies per pathway. Leveraging meta-omics data from eight spontaneous sourdough samples, we constructed a synthetic microbial community, SDG, and compared it to SMC-SD43, a community reconstructed from scratch using microbial composition data from its natural counterpart. Depletion of a single player did not significantly impact the KEGG number of dominant species in SDG, while subdominant and satellite species exhibited fluctuations, highlighting their unique contributions. SDG demonstrated broader transcriptome redundancy compared to SMC-SD43. Long-term back slopping experiments revealed the stability of SDG's volatilome profile, while SMC-SD43 experienced loss of taxon members. Collectively, dominant, subdominant, and satellite players ensured gene and transcript redundancy in SDG. This study showcases the metabolic contributions of individual players in sourdough communities through the reconstruction of synthetic communities from natural sourdoughs. Our findings emphasize the importance of including dominant, subdominant, and satellite players in sourdough metacommunities to ensure resilience and optimal performance. Overall, our study lays theoretical groundwork for guiding food fermentations toward enhanced stability and functionality. While the continuation of this work will lead to the proof of concept.

PS2-S11-PP04 Cryopreservation of microbial consortia isolated from Apulian table olives: Effects on vitality and functional potential

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Microorganisms are precious and irreplaceable biological resources for the green, sustainable transition of food systems. The characterization and preservation of microbial communities are key aspects for maintaining specific ecosystems of interest for food fermentation, promoting biotechnological innovation and supporting research purposes. Recently, microbiologists have focused their efforts on preserving whole microbiomes, but few studies have been conducted to optimize the storage conditions of microbial consortia by preserving their viability and functional potential. Here, we used microbiomes associated with fermented table olives as model matrices because of microbial complexity and for their importance in the Mediterranean area. This study aims to preserve the microbiota isolated from typical Apulian table olives cv Leccino, and to evaluate the effectiveness of a cryopreservation protocol by using glycerol and DMSO as cryoprotectants at -135 °C storing temperature. The microbial population was studied before and after a fixed period of storage using a culture-dependent approach and RNA-based metabarcoding analysis. Moreover, the metabolic profile was evaluated using Biolog Ecoplate. Preliminary results indicated that after one month of cryopreservation, the viability of the whole microbial consortium decreased by 1 logarithmic unit regardless of the cryoprotectant used. No significant changes in the metabolic profile before and after cryopreservation were observed, except for y-Amino Butyric acid. Also, metabarcoding analysis showed no significant differences in relative aboundances after a short period of storage. Results confirmed the proper preservation of the microbial consortium and its functionality after a short-term period of storage. Further monitoring of vitality, metabolic profile, and microbiota diversity is necessary to provide evidence of the effectiveness of the applied cryopreservation protocol in the long-term storage period.

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PS2-S11-PP05 Fermented sausage microbiome: Investigation, storage and exploitation

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Meat fermentation is driven by a complex succession of microbial consortia as well as by biochemical transformations able to produce a variety of metabolites responsible for flavours, odors and texture (Srinivas et al., 2022). Unfortunately, in modern sausages production, the increasing use of starter culture to guarantee safety and standardize the properties of the final product, have reduced the peculiar organoleptic characteristics of spontaneous fermented sausages, leading to a loss of typicality and a decrease in biodiversity (Cocolin et al., 2001; Franciosa et al., 2022; FAO,1999).Consequently, knowledge and conservation of the microbial biodiversity from spontaneous fermented sausages play an important role in protecting typical national products in order to maintaining quality, biodiversity and sustainability. In this study we have characterized the community structure, diversity, activity and interactions of fermented sausages microbiomes and optimized the conditions and protocols for longterm preservation and their propagation. Forty-five spontaneously fermented sausages from different regions of Italy were collected and were subjected to microbiological and metagenomic analysis. Then, besed on sensory analysis, the best 3 samples were chosen for the optimization of conditions and protocols for long-term preservation and propagation of microbiomes. Indeed, from each chosen sample, 3 preservation forms were placed at -80°C with the addition of cryoprotectant. Furthermore, the 3 preserved forms were subjected to microbiological, metagenomic and metabolomic analysis after storage. Concerning the optimization of conditions and protocols for microbiome propagation, the thawed storage form was propagated in different media. After incubation at 30°C, the same analysis was carried out at 24 and 48 hours. The results of our first trial for the propagation of microbiome of fermented sausage which was stored for 4 months showed that the fermented sausage, the first 10- fold serial dilution propagated in the 3 media for 24 hours is the best methodology to reach a bacterial load similar to that prior to storage.

Poster Session 2: Connectivity of microbiomes in the food system

PS2-S12-PP01 Zonation of the *Vitis vinifera* microbiome in Vino Nobile di Montepulciano PDO production area

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Relationship between wine and its territory is defined by the concept of terroir, including all factors explaining product attributes, and regulated in EU through the Protected Designation of Origin (PDO). Among the terroir components, microbiome linked with the location was reported to be relevant for the winemaking process, but little is known about its variations at local spatial scale and its role in plant growth promoting (PGP) functions.

We studied differences in microbiome attributes at soil-root interface of Vitis vinifera samples from different subzones (Additional Geographical Units, AGUs) within the area "Consorzio del Vino Nobile di Montepulciano DOCG," Tuscany, Italy.

16S rRNA and ITS genes metabarcoding was applied for soil and rhizosphere characterization to investigate microbial signatures of the Montepulciano territory and other vineyards worldwide, and between AGUs. Shotgun metagenomics was applied to clarify the microbial ability to support plant growth due to PGP functions.

We observed spatial distance effects in composition of microbial communities in the soil ecosystem at different geographical scale when comparing studied terroirs and other vineyards globally. This was confirmed when investigating differences in the rhizosphere microbiomes of Montepulciano AGUs, where geographical separation was retained regardless other agricultural parameters. We identified discriminant microbial genera between the AGUs, and we tested their potential to support plant growth. We identified at least one of the investigated PGP functions in at least one of the discriminant bacterial genomes, and four of the six investigated fungal genera resulted with a positive match for at least one of PGP functions.

We studied PGP functions in metagenome assembled genomes (MAGs) from a subset of samples to increase resolution of the microbial components in the different AGUs and to confirm that PGP functions of the terroir-associated microbiome is retained despite variations at a fine spatial scale.

We discovered differences of the microbiome of the Montepulciano vineyards from those worldwide, and within the consortium, based on sampling location with AGUs characterized by an associated microbiome which supports plant growth, despite its taxonomic structure.

Differences at local scale could be related with changes in the features of wine, characterizing microbial counterpart of terroir as a relevant point when dealing with products linked to territoriality.

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