MICROBIAL DIVERSITY 2025

EMPOWERING THE ECOLOGICAL TRANSITION

SIMTREA

SAPIENZA
UNIVERSITÀ DI ROMA

8TH INTERNATIONAL CONFERENCE ON MICROBIAL DIVERSITY

MICROBIAL DIVERSITY FOR EMPOWERING THE ECOLOGICAL TRANSITION:

RESEARCH, INNOVATION, AND TECHNOLOGICAL TRANSFER

23-26 September 2025
Rome, Italy

Book of Abstract



MICROBIAL DIVERSITY 2025

EMPOWERING THE ECOLOGICAL TRANSITION

SIMTREA

SAPIENZA UNIVERSITÀ DI ROMA

SCIENTIFIC PROGRAMME

TUESDAY 23 September 2025

16:00 Registration

16:45 **Institutional Greetings**
Chair: ***Carlo G. Rizzello***

Rosalba Lanciotti - President of SIMTREA
Antonella Polimeni - Rector of the Sapienza University of Rome
Maria Sabrina Sarto - Deputy Rector for Research, Sapienza University of Rome
Cristina Di Domizio - Agrifood National Cluster CL.A.N.
Daniele Rossi - Copa-Cogeca, FoodDrinkEurope

17:15 **Opening Lecture**
From single bacterial cells to microbial communities: a round trip to unlock the secrets and technological significance of microbial ecosystems characteristic of dairy niches – The Natural Whey Starter
Erasmo Neviani, University of Parma, Italy

18:00 WELCOME CEREMONY
featuring a selection of traditional baked goods from the Consorzio per la Tutela del Lievito Madre da Rinfresco





WEDNESDAY 24 September 2025

08:30 Registration

SESSION 1

SYSTEMS MICROBIOLOGY, FUNCTIONAL GENOMICS, GUT MICROBIOTA

Chairpersons: **Marco Gobbetti, Luca Cocolin, Alan Walker**

09:00 **Plenary lecture**

The human gut microbiota: Hopes, hypes and prospects for therapeutic interventions

***Alan Walker**, University of Aberdeen, United Kingdom*

Genome-wide association analysis and metabolic profiling reveal the high complexity of salt tolerance polymorphism in the nitrogen-fixing *Sinorhizobium meliloti* - **Agnese Bellabarba**

Beneficial effects of selected food-associated *Lactiplantibacillus plantarum* on social behaviors, intestinal permeability and gut microbiota in a genetic mouse model of autism spectrum disorders - **Roberta Prete**

A novel plant-based food to make the benefits of the Mediterranean diet accessible to non-adherent people - **Andrea Polo**

Evaluation of a BSH-positive probiotic strain using the SHIME® model: insights into bile acid metabolism and microbiota modulation of mild hypercholesterolemic donor - **Amanda Vaccalluzzo**

11:00 **COFFEE BREAK & POSTER SESSION**

Enduring the cold: molecular strategies of *Mrakia gelida* under temperature stress - **Daniele Andreani**

Impact of chitosan–TPP nanoparticles loaded with olive pomace extract on gut microbiota: an *in vitro* SHIME® study - **Martina Ben**

Development of a comprehensive cheese metagenome catalogue reveals potential markers of origin and quality – **Raffaele Magliulo**

Application of new genomic techniques in *Bifidobacterium animalis*: removing tetracycline resistance while preserving probiotic properties - **Marianna Bozzetti**

Seasonal variation as the main driver of gut microbiota composition in *Apis mellifera* from the Natural Park of Mount Conero (central Italy) - **Andrea Marcelli**

New functional food effects on gut microbiota-related wellness: The multi-unit *in vitro* colon model to evaluate gut health - **Pamela Vernocchi**

12:45 **LUNCH**

featuring a selection of traditional Italian cured meats from CLAI Cooperative





SESSION 2

MICROBIAL INTERACTIONS, FOOD PROCESSING AND FUTURE FOODS

Chairpersons: ***Pier Sandro Cocconcelli, Rosalba Lanciotti, Eugenio Parente***

14:00 **Plenary lecture**

Advances in the methods for the inference of microbial associations in microbiomes

Eugenio Parente, University of Basilicata, Italy

A multi-omics approach to unravel microbial and metabolic drivers of biogenic amine formation in traditional cheeses - **Leonardo Mancini**

Functional traits of *Propionibacterium freudenreichii* and its lactic acid bacteria consortia as starters for faba bean fermentation - **Rossana Coda**

Promoting Innovation of ferMENTed fOods (PIMENTO): the COST action CA20128 outcomes - **Vittorio Capozzi**

Exploiting the potential of *Weissella* and *Periweissella* strains: new insights and novel applications in functional food design - **Marco Montemurro**

16:00 **COFFEE BREAK & POSTER SESSION**

From North to South: microbial profiles and ecological characteristics of Italian fermented sausages - **Irene Franciosa**

Synergistic application of microbial fermentation and High-Pressure Homogenization for the valorisation of brewer's spent grains into functional food ingredients - **Margherita D'Alessandro**

Another brick to understand the fructophilic beahviour of *Hanseniaspora valbyensis* - **Rosangela Limongelli**

Advancing "Ewiss" cheese production and strengthening its geographical identity through the use of selected autochthonous lactic and propionic acid bacteria - **Giuliana Garofalo**

Phage-Host coevolution: a strategy for improving lytic bacteriophage efficacy against STEC in food systems - **Nicola Mangieri**

Exploring yeasts as novel natural biopreservatives: antibacterial potential against foodborne pathogens - **Laura Moretti**

Variability of insect associate microbiome as function of rearing substrate and production scale: a case study on black soldier fly larvae reared on food-related side-streams - **Giacomo Rossi**

Antimicrobial and antibiofilm effects of nanoemulsions essential oils to control pathogen in cheese - **Fabrizio Cappa**

Microbiome mapping in the meat food chain: from farm to fork - **Asim Ur Rahman**

Whole-genome sequencing of a vineyard-related *S. kudriavzevii* strain reveals links to the hybrid population with *S. cerevisiae* - **Jacopo Sica**

20:30 **GALA DINNER**

Sapienza Botanical Garden





THURSDAY 25 September 2025

SESSION 3

SOIL AND PLANT MICROBIOTA: ROOTING FOR SUSTAINABLE AGRICULTURE

Chairpersons: ***Diana Di Gioia, Olimpia Pepe, Angela Sessitsch***

09:00 **Plenary lecture**

The plant microbiome for sustainability

Angela Sessitsch, AIT Austrian Institute of Technology, Austria

Pyrroloquinoline quinone (PQQ)-producing *Arthrobacter* sp. D4 benefits peanut growth, induces antioxidant response and favourably modulates the rhizobacterial community: A prospective strategy towards antioxidant agriculture - **Aditi Buch**

Effects of municipal solid waste (MSW)-derived compost on the vineyard soil microbiota - **Massimiliano Cardinale**

Selection and evaluation of salt-tolerant Plant Growth Promoting Bacteria from halophyte plants for sustainable agriculture - **Angela Racioppo**

A simplified holobiont system revealed *Rhizobium* sp. GR12 as a possible versatile biofertilizer in grapevine under different abiotic stresses - **Francesca Mapelli**

11.00 **COFFEE BREAK & POSTER SESSION**

Enhancing agricultural sustainability: selection of *Rhizobium sullae* strains for optimized nitrogen fixation from populations of *Sulla coronaria* in Sicily - **Monica Auteri**

Monitoring of mobile genetic elements associated with AMR and VF in microbial biostimulants: a safety assessment based on in silico data and scientific literature - **Gabriele Bellotti**

Unravelling the endophytic bacterial microbiota of tomato roots: a step towards sustainable agriculture - **Francesco Maria Fagnano**

Genetic and functional diversity of arbuscular mycorrhizal fungal spore associated bacteria occurring in Mediterranean sand dunes - **Arianna Grassi**

Exploring the potential of lactic acid fermentation to produce innovative biostimulants - **Jasmine Hadj Saadoun**

Characterization of stable poultry manure: microbial composition and effects on crop growth and yield - **Elia Pagliarini**

Harnessing native *Bacillus* sp. for sustainable enhancement of saffron and other vegetatively propagated crops: A microbial strategy for ecological transition - **Nitika Sharma**

12:45 **LUNCH**





SESSION 4

MICROBIAL ECOSYSTEMS, ENVIRONMENTAL SUSTAINABILITY

Chairpersons: ***Daniele Daffonchio, Monica Agnolucci, Per Halkjær Nielsen***

14:00 **Plenary lecture**

Novel insights into the microbial diversity and ecology of global wastewater treatment and resource recovery systems

***Per Halkjær** Nielsen*, Aalborg University, Denmark

Isolation and molecular characterization of metabolites produced by an Antarctic fungal strain - **Benedetta Fongaro**

Impact of mowing frequency on soil microbial diversity of urban roadside lawns - **Anna Markowicz**

Fungi and plastisphere: can this biodiversity hide biotechnological powerful biocatalysts? - **Federica Spina**

Rebuilding belowground biodiversity: AMF and cover plants drive VOC regulation in urban soil restoration - **Maria Alexandra Cucu**

16.00 **COFFEE BREAK & POSTER SESSION**

Spontaneous bio-recycling: recovering bioactive molecules through endogenous microbial maceration of hemp residues - **Angela Conti**

Development of a biotechnological method for enhanced oil recovery from exhausted oilfields on the Absheron Peninsula - **Saida Aliyeva**

Study of mycobiota in honeybee ecosystem through fermentation monitoring - **Silvia Gattucci**

Harnessing novel indigenous bacterial potential for biodegradation of end-of-life tires using immobilized bioreactor - **Shobha Mule**

Fungal diversity, a nature-based solution for rare elements recovery: investigation on interactions between fungal strains from culture collections and elements of strategic interest - **Veronica Spinelli**

Biological disinfection of domestic wastewater using moving bed biofilm reactor with saturated sand filter and its metagenomic analysis - **Sanam Prabhudesai**

18:00 **Guided tour of SAPIENZA MUSEUMS**



FRIDAY 26 September 2025

SESSION 5

SCALING UP MICROBIAL SOLUTIONS FOR THE INDUSTRY

Chairpersons: ***Danilo Ercolini, Carlo G. Rizzello, Stefan Cappelle***

9:00 **Plenary lecture**

Food fermentation for good: scaling tradition

Stefan Cappelle, Puratos, Belgium

A molecular method for rapid detection of a *Metschnikowia pulcherrima* strain during grapevine biocontrol in industrial withering conditions - **Tiziana Nardi**

From waste to bioplastics: integrated approaches for polyhydroxyalkanoates (PHAs) production using wild type and engineered microorganisms - **Marina Basaglia**

Microbial biodiversity of traditional cheeses and natural dairy environments as drivers of food innovation - **Federico Baruzzi**

Sequential anaerobic–aerobic bioremediation of chlorinated ethenes in a complex contaminated site - **Sarah Zecchin**

11:00 **COFFEE BREAK & POSTER SESSION**

with a selection of Barilla specialities

From lab to industry: scalable production of alkaline proteases from *Bacillus licheniformis* for sustainable biostimulant development - **Beatrice Farda**

Partial grains germination and targeted lactic acid bacteria fermentation: a powerful duo to enhance nutritional and functional quality in the baking industry - **Giuseppe Perri**

Characterization of bacterial cellulose produced by *Komagataeibacter* sp. using agri-food byproducts as cost-effective substrates - **Joel Armando Njieukam**

Paper-based packaging and microbial ecosystems: a circular approach to food safety and environmental sustainability - **Gianluca Castellini**

Engineered yeasts for sustainable dsRNA production: molecular tool development and fermentation optimization - **Alessandra Di Canito**

Microbial diversity and dynamics of fruit-flavored traditional yoghurt ‘Ergo’ in Southern Ethiopia - **Habtamu Hawaz Taffese**

12:45 **CLOSING CEREMONY AND AWARDS**

13:15 **LIGHT LUNCH**

14:00 **SIMTREA MEETING**

From single bacterial cells to microbial communities: a round trip to unlock the secrets and technological significance of microbial ecosystems characteristic of dairy niches – The Natural Whey Starter

Neviani Erasmo

University of Parma, Italy

Natural whey starter (NWS) plays a key role in the production of Grana Padano and Parmigiano Reggiano cheeses, shaping the microbiota of the curd and serves as engine of the metabolism involved in cheese ripening.

NWS is complex microbial community, versatile and robust, able to perform specific activities and better tolerate environmental shifts compared to pure cultures used in other cheese production. It is possible to speculate that the management of this dynamic ecosystem should consider it as a super-organism, consisting of the sums of microbial metabolisms and interactions among individual microbes. The biological complexity and biodiversity present in NWS guarantee its ability to adapt to artisanal production technologies based on its intrinsic capacity to evolve in relation to external factors.

Despite the results provided by previous studies, the composition of these natural microbial cultures shaped by the selective pressure of dairy processing is still not entirely clear, and further studies will be necessary to understand the role of each individual actor, and, most importantly, the bacterial interactions within the context of complex ecosystem communities.

This Lecture seeks to summarize the knowledge acquired over the last 50 years on the "secrets" of NWS. At the same time, it highlights the questions that remain unanswered. We are only beginning to scratch the surface of understanding these fascinating microbial worlds After working to isolate individual microbial cells and study the mechanisms of their functioning and development, it is time to embark on a backward journey "from the small to the complex" for a better understanding of complex microbial ecosystems and their application potential.

The aim of this contribute is to deepen our understanding of the role of microbial communities in nature and how they develop and evolve.

Session 1

Systems microbiology, Functional genomics, Gut microbiota

Plenary Lecture

The human gut microbiota: Hopes, hypes and prospects for therapeutic interventions

Walker Alan

University of Aberdeen, United Kingdom

Public, academic and commercial interest in the human microbiome has increased exponentially over the last two decades. There is now a concerted effort, involving researchers around the world, to manipulate the microbiome for therapeutic purposes. There have been a number of encouraging advances, but much work remains to be carried out before we truly understand the roles the microbiome plays in health and in disease.

In this presentation, I will summarise work we have done to better understand how the microbiome responds to dietary interventions, and to identify gut microbes with the potential to be used as live biotherapeutic products.

Finally, although it has been a truly exciting time for microbiome research, the field has also attracted a significant amount of overhype. I will therefore also highlight efforts to improve rigour in microbiome research, and finish with some perspectives on challenges that remain to be overcome before we can reproducibly alter the microbiome in beneficial ways.

Genome-wide association analysis and metabolic profiling reveal the high complexity of salt tolerance polymorphism in the nitrogen-fixing *Sinorhizobium meliloti*

[1]Bellabarba Agnese, [1]Fagorzi Camilla, [1]Bacci Giovanni, [1]Decorosi Francesca, [1]Checcucci Alice, [1]Pacini Gaio Cesare, [2]Bekki Abdelkader, [1]Mengoni Alessio, [3]Pini Francesco, [1]Viti Carlo

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Salinity is nowadays one of the largest constraints in agriculture impairing the legume-rhizobia symbiosis and in turn biological nitrogen fixation. Selecting NaCl-tolerant strains and improving native strains fitness under salt stress are key points to ameliorate rhizobium-legume symbiosis effectiveness under salt stress. Understanding the genetic basis of this complex phenotype offers promising targets for the selection of symbiotic systems adapted for saline soils. To identify genes that might be responsible for salt resistance in *S. meliloti* strains, we performed a k-mer-based GWA analysis on a pool of *de novo*-sequenced and well-characterized strains. In particular, the genome sequences of strains and the phenotypic matrix, gained with a thorough *in vitro* screening at progressively higher NaCl concentrations, were used to pinpoint specific k- mers significantly associated with salt-tolerance phenotype. The retrieved k-mers allowed us to identify several loci associated with the variability in salt tolerance and involved in various processes, including cell wall organization (peptidoglycan transport and degradation), LPS biosynthesis, quorum sensing and carbohydrate transport and metabolism (as galactose metabolism, sorbitol biosynthesis, trehalose metabolic process, and cyclic-glucan synthesis). To confirm the involvement of carbohydrates, metabolic profiling of salt-tolerant and salt-sensitive strains were recorded on different carbon sources in presence and absence of salt. Salt-tolerant strains showed broader abilities in carbon sources exploitation under salt stress, showing comparable kinetics in both conditions on L-arabinose, D-arabinose, D-galactose and N-acetyl-D- Glucosamine sources, and confirming some key genetic determinants previously detected. Our study's findings provided new insights into the knowledge of the mechanisms of salt tolerance in *S. meliloti*, underscoring the multifaceted nature of salt tolerance in *S. meliloti*.

Beneficial effects of selected food-associated *Lactiplantibacillus plantarum* on social behaviors, intestinal permeability and gut microbiota in a genetic mouse model of autism spectrum disorders

[1]Prete Roberta, [2]Di Domenico Marco, [1]Sabatini Giusi, [1]Boccadoro Ilenia, [3]Serafini Federica, [4]Ricceri Laura, [3]De Jaco Antonella, [2]Cammà Cesare, [1]Battista Natalia, [1]Corsetti Aldo

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Several studies demonstrated that neurodevelopmental conditions, such as autism spectrum disorders (ASD), are significantly related to the composition of the gut microbiota whose alterations can influence gastrointestinal (gut dysbiosis and impaired intestinal permeability) and neurobehavioral symptoms. The use of probiotics is emerging as promising alternative approach to ameliorate ASD-associated dysfunctions. This study evaluated whether the administration of probiotic candidate, *Lactiplantibacillus plantarum* (LP), previously characterized for anti-inflammatory activity, could positively modulate the intestinal microbiota and alleviating ASD-associated intestinal and social impairment in a genetic ASD mouse model. The Neuroligin3 (NLGN3) R451C knock-in (KI) mouse model, showing alterations in the gut microbiome and gastrointestinal dysfunction, was employed as ASD model. Wildtype (WT) and KI mice were fed with selected LP strains (10^9 CFU/mouse/day) for 5-weeks. Following behavioral testing, mice were sacrificed, and gut and fecal samples were collected. The V3–V4 hypervariable regions of the 16S rRNA gene were targeted for taxonomic profiling of the microbial communities by NGS. Intestinal permeability was assessed by determining the mRNA levels of genes encoding tight junctions' proteins. Results showed a significant impact of LP on improving social behaviors, restoring intestinal permeability and shaping gut microbiota in KI-LP mice. NGS analysis revealed significant differences in alpha and beta diversity comparing LP-groups leading to a normalized taxa distribution, with decreased *Firmicutes*/*Bacteroidetes* ratio in LP-KI mice linked to a relative major increase of some gut-beneficial taxa. LP intake also played a key role in KI mice fostering *Akkermansia muciniphila*. This study might open new frontiers for the development of dietary therapies for mitigating ASD symptoms; the ultimate translational goal is to develop functional food enriched with specific mixtures of LP strains as targeted and personalized dietary intervention, providing an alternative biotherapeutic strategy in ASD.

A novel plant-based food to make the benefits of the Mediterranean diet accessible to non-adherent people

[1]Polo Andrea, [2]Calabrese Francesco Maria, [1]Tlais Ali Zein Alabiden, [3]Ferrocino Ilario, [4]De Filippis Francesca, [2]Celano Giuseppe, [4]Valentino Vincenzo, [3]Cocolin Luca Simone, [4] Ercolini Danilo, [2] De Angelis Maria

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The Mediterranean Diet (MD) tangibly impacts on human health, but the access to this virtuous dietary habit is difficult for a considerable part of the population. In response to this challenge, we selected ingredients with high content of bioactive components typical of MD and designed and manufactured a MD-based food. Its effect on human gut microbiota, microbiome and metabolome were explored in comparison to placebo by feeding a Twin M-SHIME. The fecal donor used for the inoculation of the Twin M-SHIME was chosen within a cohort of individuals showing traits of low adherence to MD.

The administration of MD-based food increased the abundance of numerous taxa, almost all having the potential to exert beneficial activities. Some detrimental taxa increased their abundance with the administration of the placebo but remained under control with MD-based food. The reshape of the microbiota reflected on microbiome changes. We observed increased abundances of genes responsible for colanic acid biosynthesis, a microbial metabolite implicated in longevity and healthy aging. The abundance of genes involved in L-valine pathway decreased, while the intake of MD-based food boosted the abundance of genes responsible for the carbohydrate metabolism. MD-based food temporarily increased the synthesis of SCFAs, which reflected the increased abundance of genes responsible for butyrate synthesis and fiber degradation. MD-based food modulated the synthesis of VOCs, with particular reference to esters derived from medium- and long-chain fatty acids, and polyunsaturated fatty acids.

Overall, the intake of this novel MD-based food *in vitro* orchestrated positive changes in the gut microbiota, microbiome, and metabolome, making possible for people who do not adhere to MD the opportunity to access dietary benefits.

Evaluation of a BSH-positive probiotic strain using the SHIME® model: insights into bile acid metabolism and microbiota modulation of mild hypercholesterolemic donor

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Hypercholesterolaemia is a major risk factor involved in chronic inflammatory diseases, often linked to poor dietary habits and genetic predisposition. Among various intervention strategies, probiotic strains have been proposed as modulators of cholesterol metabolism, particularly through bile acid (BA) biotransformation mediated by the enzymatic activity of Bile Salt Hydrolase (BSH). This study investigated the functional potential of a *bsh*-positive probiotic strain using the *in vitro* Simulator of the Human Intestinal Microbial Ecosystem (SHIME®). In the first phase, a SHIME® upperGIT model was used to simulate the stomach down to the colon, evaluating strain survival (culture-based methods), *bsh* gene expression (RT-qPCR) and bile acid deconjugation (UHPLC/HR-MS). The results demonstrated high survival through GIT and a progressive downregulation of bsh gene expression from the small intestine to the colon. Bile acid profiling showed that strain supplementation reduced levels of conjugated BA, such as taurocholic, taurodeoxycholic, glycolic and glycodeoxycholic acids, in the small intestine and improved deconjugation efficiency in colonic compartments. The second phase involved a long-term Mucosal SHIME® (M-SHIME®) model simulating the intestinal microbiota of a mildly hypercholesterolaemic donor. The *bsh*-positive strain was administered daily for three weeks, followed by a ten-day washout. Treated and control units included lumen and mucus compartments, which were sampled for metagenetic, metabolomic, and physicochemical analyses (lactate, ammonia, SCFAs, BCFAs). No significant differences in α-diversity were observed; however, treatment induced a reduction in *Rikenellaceae* and *Ruminococcaceae* in the lumen, and a significant decrease in *Enterobacteriaceae* in the mucus. Metabolomic differences between treated and control units were also noted. In conclusion, the applied method highlights the promising effect of the *bsh*-positive probiotic strain on the bile acid metabolism modulation, in the context of mild hypercholesterolaemia.

Enduring the cold: molecular strategies of *Mrakia gelida* under temperature stress

Andreani Daniele, Turchetti Benedetta, Marconi Gianpiero, Albertini Emidio, Buzzini Pietro

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Understanding how psychrophilic yeasts cope with low temperatures is a growing area of interest, both for its ecological relevance and for its biotechnological potential. On one hand, these organisms play an important role in organic matter turnover in cold ecosystems; on the other, their enzymes may offer valuable applications in industrial processes that require activity at low temperatures. In light of global climate change, gaining insight into these mechanisms becomes even more relevant.

In this study, three strains of *Mrakia gelida* were cultivated in liquid YPD medium at 15°C (optimal growth), then subjected to cold stress at 4°C, and finally returned to 15°C to assess recovery. Samples from each phase were collected for RNA extraction and RNA-seq analysis to investigate the transcriptomic responses to temperature shifts. Preliminary findings identified a set of genes that respond transiently to cold-stress and suggests temporary suppression of biosynthetic activity and energy-intensive processes. Moreover, it was observed a metabolic reprogramming to maintain ATP and redox balance, as well as enhanced signal transduction for temperature sensing, transcriptional control, and chromosomal stabilization. These findings suggest that *M. gelida* utilizes diverse strategies to preserve cellular homeostasis and metabolic flexibility during cold stress. The results enhance our knowledge of psychrophilic yeast biology and may contribute to future biotechnological advancements, including the development of enzymes and other products tailored for cold environments.

Impact of chitosan–TPP nanoparticles loaded with olive pomace extract on gut microbiota: an *in vitro* SHIME® study

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Olive pomace (OP), the solid residue left after olive oil process production, contains high levels of biofunctional compounds, prominently phenolics, with well-documented health-promoting properties. However, its underutilisation fails to harness its potential, resulting in the wastage of a valuable food by- product. The effective exploitation of OP phenolics is further hindered by their inherent susceptibility to degradation. To address these limitations, we developed a novel nanoencapsulated hydroalcoholic OP extract embedded within a chitosan matrix crosslinked with tripolyphosphate (OPFE-lyo-NPs), a formulation designed to protect bioactive compounds and enhance their targeted delivery to the gut. Then, the effects of OPFE-lyo-NPs on gut microbiota composition and activity were investigated, for the first time, using the Simulator of the Human Intestinal Microbial Ecosystem (SHIME®), the most valid complement to *in vivo* studies.

Blank (lyo-NPs) and OPFE-lyo-NPs were prepared via ionic gelation, exploiting high-pressure homogenisation and supplemented to the SHIME®. Microbial profiling was performed via 16S rRNA gene metabarcoding, while the metabolic response was monitored via short-chain fatty acids (SCFAs) quantification through HPLC-UV-vis chromatography. Administration of OPFE-lyo-NPs significantly enhanced SCFAs production, particularly butyrate, and induced a potentially beneficial modulation of the gut microbial community. The treatment promoted the growth of several health-associated taxa and supported a more butyrogenic microbiota, suggesting potential benefits for host metabolic and intestinal health.

Our findings demonstrate that chitosan-based nanoparticles serve not only as effective carriers for olive- derived phenolics but also as direct modulators of the gut microbiota, underscoring their promise as microbiota-targeted nutraceuticals and advancing the sustainable valorisation of food by-products.

Development of a comprehensive cheese metagenome catalogue reveals potential markers of origin and quality

[1]Magliulo Raffaele, [2]Walsh Liam, [1]Valentino Vincenzo, [2]O'sullivan Orla, [2]Cotter Paul, [1]Ercolini Danilo, [1]De Filippis Francesca

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Cheese microbiomes shape unique signatures reflecting diverse manufacturing, geographical origins, and potential health benefits. Yet, their diversity and functional links to *terroir* and bioactive potential remain underexplored. High–throughput shotgun metagenomic sequencing raw reads from curatedFoodMetagenomicData (*n*=1044), publicly available dataset (*n*=233), and newly sequenced Protected Designation of Origin and Protected Geographical Indication cheeses (*n*=316) were merged and analysed. Furthermore, a metadata collection and standardisation (*N*metadata=18) allowed the harmonisation of diverse information about cheeses. Thus, a comprehensive metadata–curated catalogue of cheese microbiomes by collecting a total of 1,593 shotgun metagenomic samples was built. Our findings revealed different values of α– and β–diversity depending on the metadata considered, as well as different distribution patterns of microbes and key functional genes associated with flavour, quality, and health benefits. Explainable machine learning models further elucidated the cheese microbiomes classification among metadata, also suggesting potential biomarkers. Additionally, phylogenetic analyses were employed to investigate strain–level microbial diversity, while functional phylogenomic approaches linked microbial taxa to functional cheese patterns. In summary, our analysis yielded 12,579 metagenome–assembled genomes, significantly expanding existing cheese microbiome repositories by approximately 60%, including previously undescribed taxa. CONCLUSIONS. This curated cheese microbiome catalogue advances our understanding of cheese microbial ecology, offering novel insights into microbial contributions to cheese *terroir* and safety, and supporting future applications for precision fermentation and health–oriented food innovation.

This work was partially supported by the project DOMINO-"Harnessing the microbial potential of fermented foods for healthy and sustainable food systems."; European Union's Horizon Europe research and innovation programme under grant agreement No 101060218 and by the National Recovery and Resilience Plan (NRRP), Mission 4, Component 2, Investment 1.4 - CN_00000033 (D.M. Prot. 1034 of 17/06/2022), "National Biodiversity Future Center—NBFC."

Application of new genomic techniques in *Bifidobacterium animalis*: removing tetracycline resistance while preserving probiotic properties

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Probiotics are live microorganisms that provide health benefits when administered in sufficient quantities. Nevertheless, concerns persist regarding the potential transfer of antibiotic resistance genes to the gut microbiota. Recent advancements in new genomic techniques (NGT), in particular CRISPR-Cas technology, offer a promising solution to mitigate these risks by inactivating resistance genes. This study aims to evaluate whether a targeted modification in *tetW* gene can affect the functional and probiotic characteristics of a commercial strain of *Bifidobacterium animalis* subsp. *lactis*. The endogenous CRISPR-Cas system was employed to introduce three premature stop codons into *tetW* gene, leading to its inactivation. The minimum inhibitory concentration (MIC) of Genome Edited (GE) and parental strains were determined along with the growth kinetics in the presence of both lethal and sublethal tetracycline concentrations. To determine probiotic potential, the GE and parental strains were compared for tolerance to acid, bile salts, and osmotic stress, as well as for survival in simulated gastric and intestinal fluids. Functional assays included exopolysaccharides (EPS) production, auto-aggregation, co-aggregation with *Escherichia coli* and *Salmonella enterica*, and adhesion to Caco-2 and HT-29 cell lines. Additionally, survival with standardized INFOGEST *in vitro* human digestion static protocol was also tested.

The GE strain showed a significantly lower MIC for tetracycline compared to the parental strain, confirming successful inactivation of the resistance gene. Notably, the mutant retained comparable tolerance to acid, bile salts, and osmotic stress, as well as similar survival rates in simulated digestive fluids. No significant differences were found in EPS production, aggregation behavior or adhesion to epithelial cells. INFOGEST assays further confirmed that the GE strain's viability was equivalent to that of the wild type.

These findings demonstrate that CRISPR-Cas-mediated *tetW* gene inactivation enhances strain safety without compromising probiotic functionality, offering a promising strategy for developing next-generation probiotics with minimized antibiotic resistance risks.

Seasonal variation as the main driver of gut microbiota composition in *Apis mellifera* from the Natural Park of Mount Conero (central Italy)

Marcelli Andrea, Milanović Vesna, Rampanti Giorgia, Corsi Lorenzo, Ruschioni Sara, Aquilanti Lucia, Casavecchia Simona

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Pollinator populations, particularly bees, have experienced severe declines globally, raising ecological concerns with several species now threatened or extinct. In response, the European Commission launched the *EU Pollinators Initiative*, prioritizing improved knowledge, targeted conservation, and enhanced societal engagement.

In this context, this study aimed to assess gut microbiota diversity and health status of emergent and forager honeybees located in "Parco Naturale del Conero" (Central Italy) before and after secondary meadow recovery. The gut microbiota of *Apis mellifera* from three different farming companies was analyzed by qPCR and NGS in spring, summer, and autumn 2023, investigating potential changes in microbiota composition linked to seasonal and/or environmental changes.

NGS analysis showed that *Lactobacillus spp.*, *Gilliamella*, *Commensalibacter*, and *Snodgrassella* were the most abundant bacterial taxa. Overall, bacterial diversity was higher in forager bees and in summer. Overall, seasonal variation was the main driver of gut microbiota composition. About fungi, *Saccharomyces cerevisiae* was the most widespread taxon, peaking at 31.64% in bees in spring and nearly absent in autumn. Other abundant taxa included *Cladosporium*, *Debaryomyces hansenii*, *Hyphopichia burtonii*, and *Malassezia restricta*, again with seasonal fluctuations. Alpha diversity analysis (Shannon index) showed no significant differences by bee type, season, or farm, while beta diversity (Bray- Curtis) revealed clear seasonal separation.

In conclusion, the higher diversity of honeybee gut microbiota in summer and in forager bees is linked mainly to the broader variety of fresh nectar and pollen consumed, increased environmental exposure, and age-related social behaviors promoting microbial transmission within the colony. In contrast, limited diets and reduced social contact in winter and younger bees constrain microbiota diversity. These insights improve our understanding of honeybee microbiota ecology and support targeted pollinator health strategies.

New functional food effects on gut microbiota-related wellness: The multi-unit *in vitro* colon model to evaluate gut health

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The aim of this study was to investigate the effect of a new functional food incorporating prebiotics and probiotics, on gut microbiota (GM), after simulated digestion performed by a multi-unit *in vitro* colon model (MICODE). Two foods were produced and tested: i) Squacquerone-like cheese produced with starter Streptococcus thermophilus (control, CTRL), and supplemented with the probiotic *Lacticaseibacillus rhamnosus*, which was either subjected to high pressure homogenization (LrH) or not (Lr); and ii) fermented milk formulations stored at 6°C for 28 days, namely simple yogurt (control), fermented milk with lactobionic acid-enriched whey (LBA), fermented milk with LBA-enriched whey with or without the probiotic *Lacticaseibacillus rhamnosus* GG (LBA+LGG and LGG, respectively). Samples stratified by typology, storage time, and colonic fermentation phase, were then digested with MICODE and digests were characterized for ecological and functional profiles. Firstly, a positive GM response after cheese simulated digestion, highlighted an abundance of Bifidobacteria, Lactobacilli and Clostridium group IV in LrH samples. Metataxonomy revealed higher levels of Firmicutes and Proteobacteria ($p < 0.05$) after simulated digestion, as well as Megasphaera, Escherichia, Prevotella and Dorea. Moreover, an increase of short and medium chain fatty acids were detected by metabolomics. Overexpression of inferred KEGG metabolic pathways showed mainly fatty acids, novobiocin and amino acid metabolism. Moreover, fermented milks evidenced that the presence of LBA enhanced the growth and stability of probiotics as *Lactobacillus* and *Bifidobacterium* and starter cultures, improved the samples' antioxidant activity, and affected the volatilome profile of yogurt by enhancing propanoic and octanoic acid during storage.

Comprehensive genome profiling and characterization of bile salt hydrolase activity in two promising *Lacticaseibacillus rhamnosus* probiotic strains

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Bile salt hydrolase (BSH; EC 3.5.1.24) is a microbial enzyme involved in bile acids (BAs) deconjugation, contributing to host cholesterol metabolism, lipid absorption, and gastrointestinal tolerance to bile stress. In the present study, two *Lacticaseibacillus rhamnosus* strains (VB4 and VB1), isolated from the human microbiota, were evaluated for their probiotic potential through whole-genome sequencing, *in vitro* BAs deconjugation ability, and BAs tolerance assays. The genome assemblies of VB4 and VB1 included 2,769 and 2,704 coding sequences, respectively. Genome annotation revealed several strain-specific genes implicated in metabolism, DNA recombination, and structural biogenesis. Both strains possessed a single *bsh* gene encoding a C-N amide hydrolase, with conserved residues characteristic of the BSH core domain. Phylogenetic analysis confirmed high sequence similarity between the BSH proteins of the two strains. Functionally, VB4 exhibited higher tolerance to BAs and deconjugation capacity, particularly of both glyco- and tauro-conjugated BAs, compared to VB1, which showed a preferential deconjugation of glyco-conjugated BAs, commonly observed in BSH-active lactobacilli. Interestingly, *bsh* gene expression was induced under BAs stress in VB1 but not in VB4, indicating that BSH activity may be regulated by additional genetic or post-transcriptional mechanisms. Both strains lacked acquired antimicrobial resistance genes, supporting their safety for probiotic use. Overall, this study highlights the differential BSH- phenotypes of VB4 and VB1, suggesting that VB4 strain could be the best candidate for BAs modulation and cholesterol-lowering applications.

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Shotgun metagenomics reveals functional potential and safety- related features of dairy starters

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Shotgun metagenomics was employed to evaluate the functional potential and safety profile of three dairy starter cultures used in hard sheep's milk cheese production: two starters originated from a traditional natural *scotta-innesto* (Mix-A and Mix-B), and preserved at the Agris-BNSS microbial collection, while the third was a commercial formulation (CS). The analysis focused on reconstructing metagenome-assembled genomes (MAGs) to investigate technological functions and antimicrobial resistance genes (ARGs). A total of eight high-quality MAGs (completeness >70%, contamination <10%) were reconstructed, representing the dominant species in each starter. *Lactobacillus delbrueckii* and *Streptococcus thermophilus* were prevalent in the scotta-based starters, with minor *Enterococcus faecium*. In contrast, the commercial starter was dominated by *Lactobacillus helveticus, Lactococcus lactis*, *Streptococcus thermophilus*, and *Lactococcus cremoris*. Functional annotation revealed that starters MIX-A, MIX-B, and SC harbored 7,066, 5,189, and 11,349 coding sequences (CDSs), respectively—reflecting the higer number of different species in the commercial mix. Strain heterogeneity, assessed via CheckM, was observed in MAGs from MIX-A and MIX-B but not in SC. However, this metric offers only a preliminary estimate of strain-level diversity. Genes related to key dairy functions were detected, including those encoding proteolytic enzymes (*clp*, *pep*), lipolytic enzymes (esterases), and enzymes involved in flavor compound biosynthesis (aminotransferases, deaminases, decarboxylases, sulfur metabolism, citrate pathway). ARGs were identified using the CARD database. In scotta-based starter mix MAGs, *E. faecium* sequences (average relative abundance 0.02%) encoded efflux pumps, aminoglycoside acetyltransferases, lsa (resistance to lincosamides/streptogramins), and mrsc (macrolide resistance). In the commercial starter, MAGs from *L. lactis* and *L. cremoris* contained multidrug resistance efflux pumps and the vatB gene (streptogramin resistance). *S. thermophilus* MAGs also carried efflux-related ARGs. These results highlight shotgun metagenomics as a powerful tool for profiling both functional traits and safety aspects of dairy starter cultures.

Development of a novel psychobiotic fermented food for the modulation of gut-brain axis

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Modulation of gut-brain axis is an evolving concept to improve human health. Some strains of lactic acid bacteria (LAB) classified as psychobiotics, since they can produce neuroactive molecules (e.g., gamma- aminobutyric acid, serotonin), able to modulate mood and cognition in humans. We used comparative and functional genomic analysis on LAB genomes to identify the most widespread species in fermented foods genomic potential related to psychobiotic activities. We downloaded from the NCBI genes related to neuroactive molecules biosynthesis. LAB were isolated from > 400 traditional Italian FFs, including cheeses, sourdoughs and fermented olives. For each of food metagenomes and isolated genomes, the genes were mapped using efficient bio-tools. The most promising strains selected by genomic analysis were *in vitro* tested to neurotransmitter production and for resistance to gastro-Intestinal transit (GIT) following INFOGEST protocol. Finally, the results were statistically processed. Screening of food metagenomes identified cheeses as the most promising source of psychobiotic strains. LAB genomes revealing the presence of species-specific patterns. High gene occurrence related to the production of GABA, tryptophan, and other neuroactive metabolites belong to the species *Lactiplantibacillus plantarum, Lactiplantibacillus pentosus, Lactobacillus delbrueckii, Levilactobacillus brevis, Lentilactobacillus diolivorans and Lentilactobacillus kefiri*. The most promising strains from genomic and in-vitro analysis will be tested in a SHIME-model to develop a plant-based, yogurt-like product with psychobiotic properties.

Our results demonstrate the importance of comparative genomics to support *in vitro/vivo* tests to launch new products with benefits on mental health. This work, exploiting genomics as a crucial part of the research, has the potential to produce new microbial therapies that exploit the gut microbiota-brain axis to shape behavior, emotion and cognition.

Potential antibacterial activity of Chadian medicinal plants against antibiotic-resistant pathogens: a sustainable phytotherapy approach

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According to WHO data, approximately 80% of the global population in emerging regions relies on traditional medicine, particularly where access to allopathic healthcare is limited. Africa's exceptional biodiversity offers a valuable reservoir of medicinal plants rich in bioactive compounds that could potentially help address or mitigate antibiotic resistance, a critical global health threat, that significantly complicates the management of nosocomial infections, especially in African settings where treatment options are often scarce or ineffective. This study investigates the antibacterial potential of hydroalcoholic extracts obtained from traditional medicinal plants used in the Ndjamena region of Chad, specifically those collected in the CHU-BS area of Walia. Bacterial strains were selected based on their clinical relevance and prevalence in nosocomial infections, as reported by CHU-BS hospital records. The selected pathogens include *Staphylococcus aureus*, *Methicillin-Resistant Staphylococcus aureus*. The plant extracts were evaluated for their antibacterial activity as standalone agents. Additionally, their total polyphenol content and *in vitro* antioxidant activity were assessed. Preliminary results indicate that several extracts exhibit promising antibacterial effects against the tested pathogens. The identification of key active compounds is ongoing, contributing to the scientific validation of local phytotherapeutic practices. By combining ethnobotanical knowledge with microbiological and phytochemical analyses, this research highlights the potential of Sub-Saharan medicinal plants in the development of new antimicrobial strategies. The integration of traditional medicine into modern therapeutic approaches is discussed, with particular emphasis on safety, standardization, and long-term sustainability. These findings support the idea that traditional medicine, when scientifically validated, can offer effective and accessible tools to address the rising threat of antibiotic-resistant infections in healthcare settings.

Traditional fermented foods as a source of beneficial microbes: a meta-analysis

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Fermentation is an optimal strategy to extend the shelf-life of perishable products. Furthermore, it is a sustainable process that increases the concentration of vitamins, essential amino acids and minerals, improving the nutritional value. Also, Fermented Foods (FF) can be a vehicle of beneficial microorganisms. The purpose of this meta-analysis is to provide a worldwide map of the traditional dairy- and non-dairy FFs and of their associated microbiota, also focusing on potential beneficial outcomes for human health. Seventy- two studies describing the microbiota of traditional FFs through metataxonomic approaches were selected. Metagenomic reads from each study were downloaded from the NCBI SRA database and denoised through DADA2, then taxonomy was inferred. Furthermore, we screened the literature for studies focusing on the transfer of microorganisms from traditional fermented foods to the human gut and on their outcome for human health. Our results indicate that traditional FF are a valuable source of beneficial microorganisms, mostly belonging to the Lactic Acid Bacteria group, with probiotic activities. In addition, we highlighted that most of the currently approved probiotic strains have been firstly isolated from traditional FFs, thus emphasizing the nutritional and beneficial value of these foods for human health. Furthermore, literature suggests that probiotic strains from fermented foods might interact with the human microbiota, not only by establishing themselves, but also by competing for nutrients with potentially hazardous and pathogenic species.

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Yeast adaptation to early alcoholic fermentation under low pH: effects of bio-activators on fermentation performance, viability, and gene expression

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The initial stages of alcoholic fermentation are critical for ensuring a successful process and are particularly sensitive to environmental stressors such as high sugar concentrations, low pH, oxidative stress, and high temperatures. A rapid adaptation by *Saccharomyces cerevisiae* to these challenging conditions is essential. The present study investigated yeast adaptation during the first 48 hours of alcoholic fermentation under standard (pH 3.5) and low pH (2.9) conditions. The focus is on evaluating the impact of two commercial yeast-based bio-activators on fermentation kinetics, cell viability, and stress-related gene expression. The microbiological monitoring of *S. cerevisiae* populations was conducted through plate counts and flow cytometry, while gene expression was assessed via RT-qPCR. Fermentation kinetics were analysed through micro-fermentation trials in grape must and modified YPD medium, with weight loss and growth curves used to measure lag phase duration. Results showed that the addition of bio-activators significantly improved yeast cell viability, particularly at 14 and 24 hours post-inoculation, under both pH conditions. Low pH extended the lag phase, but this effect was mitigated by bio-activator supplementation. Gene expression analysis revealed enhanced expression of key stress-related genes, including *Sod1*(oxidative stress), *Hsp12* (general stress), and *Grx5* (redox homeostasis), especially under low pH conditions. These findings highlighted the beneficial role of bio-activators in promoting yeast adaptation during the early, stress-intensive phases of alcoholic fermentation, thereby more robust and efficient fermentations under challenging conditions.

Metagenomic analysis coupled with conventional culture-dependent approach revealed that *Bacillus cereus* is a highly prevalent pathogen in legume-based foods

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In the last decade, legume-based foods are spreading worldwide due to the recognized health benefits of increasing legume intakes. Nevertheless, scarce knowledge is available about the microbial safety of these products. Herein, we investigated the presence of pathogenic bacteria in legume-based foods by metagenomic analyses, directly and after enrichment in Bolton broth at 37°C overnight. Out of 92 products analyzed, 49 (53.3%) resulted positive for the presence of pathogenic food-borne bacteria (22 direct and 45 after enrichment). *Klebsiella pneumoniae, Clostridium spp., Escherichia coli, Aeromonas* spp., *Salmonella enterica, Listeria monocytogenes, Acinetobacter* spp., *Aliarcobacter* spp., *Shigella* spp., or *Pseudomonas* spp. were detected in 19/49 positive samples (5 direct and 14 after enrichment), while *Bacillus cereus* was detected in 42/49 positive samples (18 direct and 40 after enrichment). The high prevalence of *B. cereus* can be explained by the possibility of spore forming by this organism, which would enable it to survive processing conditions. Considering that *B. cereus* resulted the most prevalent pathogen in legume-based samples by metagenomic approach, additional conventional plating on the selective medium Brilliance Bacillus cereus agar was performed to investigate its viability. Interestingly, viable presumptive *B. cereus* (typical colonies on selective culture medium) were detected at amounts up to ca. 10^7 cfu/g in samples analyzed by direct metagenomics. Current research focuses on genotyping and characterizing the isolated Bacillus cereus using whole-genome sequencing. The metagenomics-based approach proved to be a valuable tool for assessing the microbial safety of legume- based foods, revealing *B. cereus* as the most prevalent foodborne pathogen.

Impact of interacting climate change factors on the growth of *Aspergillus carbonarius* strains and mycotoxins production on a grape-based matrix

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Mycotoxigenic fungi have their own specific temperature and humidity range for crop infection, mycotoxin production and survival, which reflects their geographical distribution and determines a gradient of mycotoxin contamination worldwide. Some species might shift their geographical distribution in response to global warming, leading to changes in the pattern of mycotoxin occurrence. Little is known on the impact that climate change (CC) may have on *Aspergillus carbonarius* and Ochratoxin A (OTA) contamination of grapes, especially in the Mediterranean region where in CC scenarios temperature are expected to increase by +2–5 °C and CO_2 from 400 up to 1000 ppm. This study examined the effect of i) current and increased temperature in the alternating 7 h dark/17 h light cycle (15–28 °C vs 18–34 °C), ii) different conditions of water availability (0.95 and 0.97 aw) and iii) existing and predicted CO_2 concentrations (400 vs 600 ppm), on growth, expression of biosynthetic genes and OTA production by two *A. carbonarius* strains ITEM 5010 and ITEM 11822, isolated from different climatic regions. The experiments, conducted on a synthetic grape- based matrix, showed that elevated CO_2 resulted in a general stimulation of growth and OTA production.
These results were also supported by the up-regulation of both structural and regulatory genes involved in the OTA biosynthesis. Our work has shown that elevated CO_2 concentration and higher temperature may result in an increased risk of OTA contamination in the wine production chain. Moreover, such results highlight that intraspecies variability exists among *A. carbonarius* strains from different geographical regions, pointed out a different resilience of tested strains to prospected climate change conditions.

Heat that hardens: how sub-lethal warming strengthens *Listeria monocytogenes*

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Climate change is a hot topic and global issue that is driving a range of environmental changes. Environmental stresses as a result of climate change may lead to novel and emerging responses from microorganisms. Increasing temperature is a common scenario that is expected and that has an impact on microorganisms through its direct effect on microbial physiology and fitness.

This study aimed to assess the impact of sub-lethal high temperatures on the heat resistance of *Listeria monocytogenes*.

Here we show that two strains of *L. monocytogenes* exposed to high temperature (47 ºC) *in vitro* were more resistant to subsequent heat treatment (60 ºC, 10 min) compared to strains grown at 37ºC.

Our results demonstrate that the increase in temperature as a result of climate change could make microorganisms more heat resistant. This may pose a challenge in food safety, particularly when pathogenic microorganisms grow in foods that are later subjected to cooking. Further studies assessing the behavior of *L. monocytogenes* in food matrices exposed to sub-lethal temperatures prior to heat treatment would be of great value.

Metabolic versatility with a cost: prediction of facultative anaerobes that are associated with diseases

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Bacteria are classified based on their oxygen requirements: aerobes, anaerobes, and facultative anaerobes. Strict aerobes require oxygen, while strict anaerobes cannot tolerate it. Facultative anaerobes can grow in both aerobic and anaerobic conditions by switching between aerobic respiration and anaerobic pathways such as fermentation or respiration using alternate electron acceptors like nitrate or fumarate. Other groups include aerotolerants, which tolerate but don't utilize oxygen and microaerophiles, which require low oxygen concentrations. Most importantly, facultative anaerobes are frequently implicated in human infections and have been proposed as biomarkers for dysbiosis and inflammation. Despite their importance, the precise characterization and prediction of facultative anaerobes remains challenging. Here, we show the prevalence of facultatives in many habitats and characterize their importance as pathogens and involvement in inflammatory diseases. Further we developed a machine learning approach to predict facultative metabolism using 203 phylogenetically diverse bacterial genomes from bacdive-DB. Features included genes, Pfam domains, Gene Ontology (GO-terms), pathways, and metabolic reactions and fluxes. Models were developed using random forest and linear regression, and cross-validated against curated phenotypes from the literature and bacdive-DB, demonstrating improved trait prediction over existing tools: bacdiveAI and ampliconTrait. Of the 65 models we developed, 48 showed high sensitivity (64–71%) against literature-curated phenotypes, surpassing bacdiveAI (40%) and ampliconTrait (20%). Against bacdive-DB phenotypes, 29 models achieved sensitivities of 58–65%, outperforming bacdiveAI (58%) and ampliconTrait (48%). Specificity improvements were modest, with 2 models exceeding benchmarks in literature validations (86–93%) and 4 models doing so in bacdive-DB validations (71–85%). Models using Pfam, GO-terms, genes, pathways and metabolic reactions achieved the highest balanced accuracy, outperforming existing tools. Metabolic network data emerged as a novel, informative feature for predicting facultative metabolism. Further validation with independent datasets and experimental testing is recommended to better understand this versatile and potentially pathogenic microbial life history trait.

A trait-based framework integrating genomics and systems biology to infer microbial life history strategies

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Microbial species rarely exist in isolation, rather, they form complex communities shaped by interspecies interactions such as mutualism, competition, and co-dependence. These interactions strongly influence microbial behavior, ecological function, and evolutionary trajectories. Understanding the physiological strategies that bacteria employ to survive, grow, and optimize fitness across diverse habitats is central to microbial ecology. Despite their ecological and biomedical importance, the fundamental principles underlying these trade-offs and their genomic signatures remain largely elusive. To address this, we developed a computational pipeline that predicts microbial life history traits directly from genomic data, enabling the inference of ecological strategies. Our pipeline integrates several publicly available tools to annotate a broad spectrum of genomic features, and systems biology approach that reconstructs genome-scale metabolic models to collectively reflect important physiological traits such as metabolic flexibility, biosynthetic capabilities, growth rate potential and among others. Following the generation of a comprehensive trait matrix, co-varying traits across species were clustered using unsupervised learning methods. Feature selection was then applied to reduce dimensionality and enhance model interpretability. Random Forest models were trained to uncover non-linear trait–genome relationships, with feature importance evaluated using Gini scores. Model accuracy and generalizability were assessed through cross- validation, and trait predictions against curated, publicly available Madin's trait database. As a case study, we applied this pipeline to a mouse gut microbiome dataset encompassing stroke and non-stroke conditions. This allowed us to explore potential shifts in microbial ecological strategies, such as altered competitiveness or stress tolerance, or enrichment of facultative anaerobes, associated with disease states. Our approach provides a scalable framework to explore microbial adaptive strategies and ecological roles across environments using only genomic data, offering new insights into microbiome function and evolution.

A half-century of change: evolutionary and functional changes of *Lactobacillus helveticus* in traditional dairy ecosystems

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Lactobacillus helveticus (LH) is a thermophilic lactic acid bacterium commonly found in natural whey starter (NWS) cultures used for the production of PDO cheeses such as Parmigiano Reggiano. This study compares LH strains isolated 50 years ago and never revitalized with newly isolated strains from the same niche. A preliminary genotypic screening using Amplified Fragment Length Polymorphism (AFLP) was conducted to compare DNA from the old, lyophilized strains with that of recent isolates collected from dairies in the same geographical area. AFLP analysis revealed that historical and contemporary strains form distinct genotypic clusters. From this collection, 16 old and 18 new strains were selected for whole-genome sequencing. Results showed signs of genomic decay over time: older strains had significantly larger genomes (2.09 ± 0.17 Mbp) and higher GC content (37.52% ± 1.19) compared to recent ones (1.95 ± 0.03 Mbp; GC 36.76% ± 0.26). Phenotypic and techno-functional analyses highlighted differences in dairy-relevant traits. Safety assessments included testing for resistance to EFSA-recommended antibiotics. Growth performance and metabolic profiles were evaluated using culture media and GENIII microplates on an OMNILOG system (Biolog, Inc.). Metabolic profiling showed varying substrate utilisation performance not only between old and new strains but also among current biotypes, underscoring the rich biodiversity within this bacterial species. This study provides a unique opportunity to explore some aspects of the evolution of LH under continuous technological and environmental pressure, revealing functional and metabolic shifts that influence microbial development and volatile compound production in dairy fermentation ecosystems.

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Modulation of the gut microbiome in a Simulator of Human Intestinal Microbial Ecosystem (SHIME) by a postbiotic derived from navy bean flour fermented with *Lacticaseibacillus paracasei* CBA L74

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Postbiotics—non-viable microbes, their components, or metabolites—are increasingly valued for health benefits like immunomodulation and anti-inflammatory effects. Legume-based matrices, such as navy beans, are promising substrates due to nutrient density and support for beneficial gut microbes. Controlled fermentation and inactivation yield functional postbiotic ingredients combining legume nutrition with fermentation-derived bioactivity. We optimized the formulation of a postbiotic product using navy bean flour fermented with *Lacticaseibacillus paracasei* CBA L74, a proprietary probiotic strain. The strain was inactivated by heat treatment. The impact of postbiotic administration on the gut microbiome was evaluated using the SHIME system, a dynamic *in vitro* model of the human gut. A 4-arm SHIME configuration was designed to evaluate two dosages of the postbiotic (1.2 g/day and 12 g/day) and the corresponding controls consisting of raw (not fermented) navy bean flour. Each treatment was administered over a two-week period. Samples from the different compartments were collected at defined time points for shotgun metagenomic sequencing and bioinformatic analysis. Consumption of the fermented navy bean postbiotic affected the gut microbiome, enhancing fiber-degrading and short chain fatty acids-producing bacteria. The higher dose (12 g/day) was associated with a greater increase in the relative abundance of *Faecalibacterium* and *Ruminococcus*, along with elevated butyrate levels, suggesting a dose-dependent modulation of the microbial activity with potential benefits for gut health. Conclusions. These findings support the potential of legume-based postbiotics as functional ingredients that modulate the gut microbiome in a health-promoting, dose-dependent manner, warranting further investigation in microbiome-targeted nutrition.

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Engineering yeast *Saccharomyces cerevisiae* into a microbial cell factory for producing equol, a beneficial soybean-derived phytoestrogen

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Phytoestrogens are plant-derived compounds characterised by a phenolic core structurally similar to 17-β-estradiol, allowing them to bind to human estrogen receptors and mimic estrogenic activity. Among phytoestrogens, equol is recognised for its potent beneficial effects, as it protects against tumour development, alleviates menopausal symptoms, and supports prostate health. However, equol is biosynthesised from the soybean metabolite daidzein through a four-step metabolic pathway encoded exclusively by specific gut microbiota, present in only approximately 30% of adults in Western populations. Consequently, up to 70% of Westerners are equol-deficient, remaining without the protective health effects associated with this phytoestrogen. To address this deficiency, we are engineering yeast *S. cerevisiae* into the first eukaryotic microbial cell factory for efficient daidzein-to-equol conversion. For this purpose, we have mined and codon-optimised relevant genes from the bacterial genomes of *Slackia*, *Adlercreutzia*, and *Eggerthella* species for expression in yeast. After introducing these genes into *S. cerevisiae* under the control of strong constitutive promoters, we evaluated their capability to convert daidzein to equol. With this work, we aim to establish a scalable yeast-based microbial cell factory for equol production, enabling broader availability of this potent phytoestrogen as a nutraceutical to improve bone, cardiovascular, and hormonal health in the general population.

Changes in gene expression in *Bacillus cereus* and *Bacillus subtilis* treated with essential oils

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Bacillus cereus and *Bacillus subtilis* are microorganisms of great food relevance, the first for its pathogenicity and the second for its spoilage properties. Essential oils (EOs) are promising natural antimicrobials, increasingly investigated for food applications. Their rich content of phenolic compounds can act against microbial cells influencing multiple factors, including membranes integrity, quorum sensing, and virulence. The expression of genes involved in these mechanisms can be modulated by stressful events, like exposure to EOs. Therefore, this study explores gene expression modification of *Bacillus cereus* 11 and *Bacillus subtilis* 58C, isolated from shelf-stable gnocchi, after exposure to sublethal concentrations of *Thymus vulgaris* and *Origanum vulgare subsp. hirtum* EOs. Gene expression dynamics were assessed using quantitative real-time RT-PCR across various exposure times (6, 12, 18, 24, and 48 hours). At the early stages, some genes were upregulated, like *spo0A* in both strains and *pbpF* and *sigB* in *B. subtilis*, suggesting activation of cell repair and communication pathways. On the other hand, most genes were downregulated at 6 hours, showing an extension of the lag phase under stress. In general, *pbpF* appeared to play a key role in stress response of *B. subtilis*, whereas in *B. cereus* virulence-associated genes (*plcR* and *nheB*) were the most involved. In most cases, gene upregulation was stronger at the highest EO concentration (0.63 µL/mL), suggesting a dose-dependent effect. The two EOs triggered different responses, even within the same bacterial strain, pointing to specific interactions with different molecular targets. Overall, the findings demonstrate that EO-induced stress primarily affects membrane integrity and quorum sensing, with strain- and EO-specific gene responses.

Lactiplantibacillus plantarum H64 and *Periweissella beninensis* LMG 25373^T: two new potential probiotics with a role in the modulation of the gut-microbiota-brain axis in the mouse model of autism Neuroligin3 R451C

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Probiotics, including species belonging to the former *Lactobacillus genus*, *Bifidobacterium*, and *Saccharomyces boulardii*, can survive the gastrointestinal tract and transiently colonize the gut, modulating local microbial ecosystems and host physiology. Their mechanisms include enhancing mucosal barrier function, modulating immune responses, producing anti-inflammatory cytokines, and inhibiting pathogens, alongside the production of beneficial metabolites and neurotransmitters. Gut microbiota is in communication with the central nervous system via the enteric nervous system, vagus nerve activation, immune signaling, and microbial metabolites such as short-chain fatty acids (SCFAs). Dysbiosis has been increasingly associated with neurological disorders, particularly Autism Spectrum Disorders (ASDs), where alterations in gut composition often correlate with behavioral symptoms. Emerging studies show that personalized probiotic interventions may restore microbiota composition and ameliorate ASDs-related behaviors. In this study, we evaluated the probiotic potential of two strains, *Lactiplantibacillus plantarum* H64 and *Periweissella beninensis*, respectively GABA- and exopolysaccharide-producing lactic acid bacteria strains. The strains were tested in a monogenic model of ASDs, the Neuroligin3 R451C knock-in mouse, known for its social deficits and neurotransmission alterations. Interestingly, behavioral analyses using the three-chamber sociability test revealed that both microbial strains successfully rescued social deficits in mutated mice, restoring sociability to wild-type values. At the molecular level, we aim to assess neurotransmitter alterations through neurochemical profiling in ASDs-relevant brain regions (prefrontal cortex, hippocampus, cerebellum), focusing on the potential restoration of the excitatory/inhibitory balance. Gut microbiota composition will be evaluated longitudinally through fecal sample analyses at key developmental stages. Additionally, plasma metabolomics will identify potential gut-to-brain signaling molecules (SCFAs, cytokines, neurotransmitters). This work highlights the potential probiotic features of these strains and targeted probiotic interventions to simultaneously address gut and brain dysfunctions in ASDs, offering promising insights for future translational therapies.

Exploring the modulatory role of sauerkraut juice on gut microbiota and its possible implications for the gut-brain axis

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Fermentation, a traditional food preservation method, enhances nutritional benefits and has been used globally for thousands of years. Research has particularly focused on fermented cabbage, known for its health-promoting properties and widespread popularity in diets across various cultures. Even though, sauerkraut, one of the most known cabbage fermentation products has been extensively used in *in vitro*/*in vivo* studies on the microbiome-gut-brain axis (MGBA), its liquid by-product, sauerkraut juice (SKJ) has received limited attention, despite its promising potential to modulate the MGBA. The study examines the effects of SKJ consumption on human gut microbiota and investigates potential metabolic responses linked to the MGBA. The dominant lactic acid bacteria (LAB) species found in SKJ were genotypically assessed and evaluated, based on their ability to survive the gastrointestinal transit. Findings determined the daily dose at 300mL/day, for the dynamic *in vitro* Mucosal-Simulator of the Human Intestinal Ecosystem (M-SHIME), which incorporates mucin-covered microcosms. Gas chromatography was employed to analyse the short-chain fatty acids (SCFAs) produced, assessing the metabolic response of the gut microbiota to the treatment. Meanwhile, a triple-quadrupole mass spectrometer was used to monitor potential release of key neurotransmitters and their precursors. While the overall microbiota composition was assessed via 16S rRNA sequencing, quantitative polymerase chain reaction (Q-PCR) was applied to observe the LAB colonisation dynamics during the M-SHIME experimentation. Preliminary results point toward robust survivability exhibited by the dominant LAB species in SKJ. The potential of these LAB to alter SCFAs profiles in a simulated gut environment suggests an enhanced metabolic activity of the local microflora. Additionally, initial findings imply the possibility that SKJ may positively affect the concentration and bioavailability of neurotransmitters and/or their precursors. This study contributes to the growing evidence that functional beverages like SKJ can modulate the gut microbiota and potentially interact with gut-brain communication pathways.

Unveiling the gut microbiome and metabolic responses of *Eisenia andrei* exposed to polymetallic-contaminated soils in an industrial area of southern Tunisia through a multi-omics methods

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The phosphate industry poses considerable environmental challenges, particularly due to the accumulation of phosphogypsum, an industrial byproduct containing heavy metals and organic pollutants. These contaminants degrade soil quality, alter microbial communities, and threaten biodiversity and human health. Earthworms such as *Eisenia andrei* are widely recognized as bioindicators of soil condition, as they exhibit measurable shifts in gut microbiota and metabolic activity in response to pollution. This study aimed to characterize the microbiota of soils from contaminated and reference sites, assess alterations in earthworm gut microbial communities following exposure to contaminated soils, explore metabolic perturbations in the earthworm gut, and integrate 16S amplicon sequencing and metabolomic datasets to better understand ecological impacts. Soil samples were collected from several sites in the industrial zone of Gabes, Southern Tunisia, selected for their varying levels of contamination. Adult *Eisenia andrei* were exposed to these soils under controlled conditions for 14 days. DNA was extracted from soil and gut samples and analyzed through 16S rRNA gene sequencing to profile bacterial and archaeal communities. Metabolomic profiling of gut extracts was performed using UHPLC-MS to detect changes in response to heavy metal exposure. Multiomics integration was carried out to explore associations between microbial taxa and metabolites. Sequencing results showed compositional changes in the gut microbiota, with an increase in *Proteobacteria* and a decline in *Clostridia* in earthworms exposed to highly contaminated soils. Metabolomic data revealed disruptions in pathways related to oxidative stress, amino acid biosynthesis, and energy metabolism. Data integration identified associations between specific microbial taxa, such as *Vibrio* spp. and *Myroides phaeus*, and metabolites like histamine, suggesting roles in physiological stress responses. Overall, this study demonstrates that polymetallic contamination influences both microbial and metabolic responses in *E. andrei*.

Dietary shift from red meat to plant-based proteins modulates the gut microbiome

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Dietary recommendations suggest increasing the intake of pulses to reduce the meat-related health risks and to ensure adequate proteins intake in a sustainable way. This work explored the effects of replacement of red meat with pulses and plant proteins on the gut microbiome. Eighty-four participants selected according to their dietary habits were randomized into three groups: control diet (HabD, n = 28), pulses diet (PulD, n = 28) and plant protein diet (PPD, n = 28). The participants consumed a tailored isoproteic and isocaloric diet, and whole microbiome sequencing was performed on faecal samples donated at baseline, mid-treatment (4 weeks), and treatment end (8 weeks).

Although the diversity of the microbial communities did not change between the experimental groups upon treatment, different health-promoting and butyrate-producing species were selected, such as *Faecalibacterium prausnitzii* and *Agathobaculum butyriciproducens*. Consistently, the functional annotation of metagenomes highlighted the enrichment of genes involved in production of short chain fatty acids including butyrate (i.e., the key energy source for gut colonocytes) in PulD and PPD upon treatment, as well as the depletion of genes linked with microbial-mediated gut inflammation.

Overall, the results suggest that a diet enriched in proteins from plant sources can modulate the gut microbiome composition and metabolic potential favoring the selection of species and metabolic traits that benefit human health.

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Performance of *Escherichia coli* O157:H7 biofilms challenged with antagonistic lactic acid bacteria

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Escherichia coli O157:H7 biofilms established on inadequately cleaned/sanitized processing facilities can be a source of subsequent food contamination, which constitutes a serious risk to public health. The food industry urgently requires effective solutions to mitigate and prevent these risks. Lactic acid bacteria (LAB) are generally recognized as safe organisms and constitute a promising eco-friendly strategy against pathogens. In the present work, studies were conducted on the behaviour of *E. coli* O157:H7 biofilm challenged with three LAB, converging on some of the mechanisms involved in this bacterial interaction by physiological, proteomic and electron microscopy evaluation. Results showed the capacity of E. coli O157:H7 NCTC 12900 strain to produce biofilm under technological conditions tested at 12°C. Interaction between *E. coli* and LAB in biofilm mode was examined using three strategies —competition, exclusion, and displacement—. The three LAB strains tested effectively reduced *E. coli* biofilms during competition and exclusion, with competition emerging as the most effective strategy. Notably, *Pediococcus pentosaceus* CRL 2145 was able to reduce E. coli biofilm also through displacement. Clusters of LAB cells surrounding damaged pathogen cells were observed by scanning electron and confocal laser scanning microscopy.

Proteomic analysis of *E. coli* revealed repression of 85 proteins involved in metabolic pathways, stress responses and biofilm maintenance. Additionally, some adhesion and virulence proteins were upregulated. This study contributes to elucidate the response of sessile *E. coli* O157:H7 when challenged with antagonistic LAB, which could constitute a new biological alternative for combating E. coli biofilms on food processing surfaces.

Exploring the microbial restoring potential of an anaerobic bacterial blend in an antibiotic-disrupted gut ecosystem *in vitro* model M-SHIME®

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Disruption of the human intestinal microbiota (dysbiosis) caused by antibiotics is an emerging health risk, impacting the onset of different clinical diseases. Faecal microbiota transplantation (FMT) can restore gut microbiota in dysbiotic patients, such as those with recurrent *Clostridioides* difficile infection. While FMT is generally safe and tolerated, its limitations have prompted to the administration of next-generation probiotics as current probiotics are ineffective against severe dysbiosis. This study evaluated a bacterial mix (BM) containing 14 anaerobic freeze-dried fecal isolates in restoring gut microbial ecosystem using *in vitro* mucosal-simulator of the human gastrointestinal tract (M-SHIME®). Dysbiosis was induced over 5-days by clindamycin, followed by 14 days of daily administration of BM or matrix control, and 10-day of wash out. Microbiota metabolism was assessed through lactate, ammonia, and short and branched chain fatty acids (SCFAs and BCFAs) concentrations, while microbiota profiles were analysed with metagenomics. Results showed stable ammonia concentrations, whereas lactate and SCFA/BCFAs production was increased at 2-weeks after the BM treatment as compared to the control. Initial analysis of metagenomic data has identified promising correlations with microbial profiles. Furthermore, we addressed the effect of dysbiotic and restored microbiota on epithelial integrity and induction of inflammatory response *in vitro* using enterocyte cell models. After two weeks, BM treated sample induced significantly lower IL-8 release from HT-29 enterocytes as compared to the control. Overall, BM treatment showed potential in alleviating microbiota dysbiosis resulting in improved restoration of microbial metabolism and less proinflammatory microbial consortia, as compared to the control treatment.

Genomic and functional insights into the Bile Salt Hydrolase activity of the food-derived *Levilactobacillus brevis* M3R3 strain

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Levilactobacillus brevis, a species with QPS status, is recognized for its probiotic potential, including bile tolerance, gut persistence, and cholesterol-lowering effect, primarily due to bile salt hydrolase (BSH) activity. Here, *L. brevis* M3R3, isolated from sourdough, was evaluated for BSH functionality through kinetic growth and bile salts (BSs) deconjugation using UHPLC/HR-MS. Genome sequencing and *in silico* analysis were performed to assess its safety and probiotic potential, and *bsh* gene expression was analyzed by qPCR. M3R3 tolerated physiological concentrations of conjugated BSs, with dose-dependent growth inhibition. Exposure to 1.0% of taurocholic and taurodeoxycholic acids reduced growth, indicating adaptative response. In a mixed BSs solution mimicking intestinal conditions, M3R3 maintained growth, despite an extended lag phase and reduced final biomass, likely due to toxic BSs accumulation. UHPLC/HR- MS confirmed complete deconjugation of glyco-conjugated BSs and partial deconjugation of the tauro- conjugated BSs. Whole-genome sequencing (2.30 Mbp, 2375 CDSs) revealed the absence of antimicrobial resistance or virulence genes, and identified the presence of two *bsh* genes (bsh2 and bsh3), both expressed under control and BS-exposed conditions. Phylogenetic analysis of 78 L. brevis BSH proteins identified three BSH clusters (I, II, and III), with M3R3 bsh2 and bsh3 genes grouped in clusters II and III, respectively. The results support M3R3 as safe, BSH-active strain with potential as functional ingredient in health-promoting applications.

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Mathematical Modeling of Onychomycosis Transmission Dynamics and the Antifungal Role of Cymbopogon citratus: A One Health Study in Benue State, Nigeria

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Onychomycosis, a fungal nail infection, significantly impairs quality of life and is increasingly linked to environmental and hygiene-related factors. This study evaluated the antifungal efficacy of *Cymbopogon citratus* (lemon grass) leaves and stems against fungi associated with onychomycosis in adults from Benue State, Nigeria. Additionally, a mathematical model was developed to simulate the transmission dynamics of onychomycosis over two years, assessing key behavioral and environmental drivers within a One Health framework.

A total of 384 infected nail samples were collected and examined. Fungal species were isolated using standard microbiological techniques, and prevalence and risk factors were determined through structured questionnaires. *C. citratus* was extracted using methanol, ethyl acetate, and hexane. Phytochemical screening and GC-MS analysis were conducted to identify active compounds. Antifungal susceptibility testing compared the efficacy of *C. citratus* extracts, essential oils, and Terbinafine. A deterministic mathematical model was constructed to evaluate disease transmission and control strategies.

The most common isolates were *Trichophyton rubrum* (70.36%), *Aspergillus* spp. (13.27%), and *Candida* spp. (8.41%). Onychomycosis was more prevalent among males aged 31–45 and urban residents. Major risk factors included occlusive footwear, long nails, and diabetes. Phytochemical analysis revealed high levels of flavonoids (5.88 mg/g) and phenols (7.67 mg/g). GC-MS identified 24 bioactive compounds, with dodecanoic acid and 1,2,3-propane exhibiting strong binding affinities. Essential oil from *C. citratus* showed superior antifungal activity compared to Terbinafine, with low MIC and MFC values against key pathogens. The mathematical model highlighted the importance of hygiene and behavioral modifications in reducing infection spread.

C. citratus, especially its essential oil, demonstrated potent antifungal properties, supporting its use as a natural, climate-resilient therapy. Integrating plant-based interventions with hygiene practices offers a sustainable One Health strategy for controlling onychomycosis.

Microbial and metabolomic profiling of fermented sorghum: assessment of its prebiotic potential in a functional gluten free bread

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Due to a large adaptability to different cultivation conditions and limited input compared to other cereals, sorghum is considered an emerging crop. Nevertheless, despite its nutritional potential, its use as food ingredient is still limited. In this study, an integrated approach based on sourdough fermentation was used to valorize sorghum and promote its application in gluten-free bread. Culture-dependent analysis revealed *Lactiplantibacillus plantarum* and *Weissella* spp. as the predominant species isolated from mature sourdough and flour, respectively. Their metabolic profile, when used as starter in fermented sorghum, was studied through chromatographic and NMR spectroscopic techniques revealing the presence of potentially functional compounds (allantoin, glutathione, γ-aminobutyric acid and 2-hydroxy-3-methylbutyric acid), whose concentration increased during fermentation in a species- or strain-specific matter.

Sorghum fermentation was also optimized to increase exopolysaccharides yield and a functional bread was produced. The presence of sorghum determined a high fiber content, often limiting factor in gluten-free products, whereas exopolysaccharides markedly improved bread glycemic index and rheological characteristics, decreasing the staling rate. After *in vitro* digestion, experimental breads were subjected to functional assessment toward the human gut microbiota, employing the robust MICODE platform. Results demonstrated that fortified sorghum bread preserves microbial eubiosis; increases the abundance of beneficial bacteria; limits opportunistic bacteria; produces bioactive organic fatty acids; reduces detrimental compounds; and generates a prebiotic effect. Overall, these results set the basis for future industrial development but, most of all, they represent an effective strategy to valorize a cereal crop whose cultivation offers numerous agronomical and environmental benefits.

Dietary microbes and food pattern typical of Mediterranean diet

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Emerging trends in scientific research highlight the contribution of dietary microbes to human health and nutrition. The Mediterranean diet (MD) has been receiving outstanding interest for its balanced nutrition intake and health effects. We provide an overview of the content in dietary microbes for different MD components, with a special focus on fermented products. The objective was to support the design of microbe- depleted and microbe-rich diets for future experimental trials. In particular, integrating experimental data and information from the scientific literature, we explored the microbiological classification of food items within the Mediterranean diet and examined the abundance and diversity of the related microorganisms. We classified Mediterranean foods based on their microbial profiles using previous information opportunely integrated with original findings using culture-dependent methods. In addition, we also evaluated some case studies that consider the combination of different ingredients in complex preparations.

This work was completed as part of the bEat project (We all eat microbes: diet as reservoir of microorganisms that preserve the ecosystem services of the human gastrointestinal microbiota) supported by the call "Progetti di Ricerca di Rilevante Interesse Nazionale (PRIN) 2022" granted by the Italian Ministry for Universities and Research (MUR) (Prot. 2022TF9AHZ, CUP G53D2300574 0006). Vittorio Capozzi was partially funded by CNR project "NUTRAGE FOE2021 DBA.AD005.225";. Mariagiovanna Fragasso is supported by the European Union NextGeneration EU [PNRR, CN00000022] within the Agritech National Research Centre for Agricultural Technologies.

Session 2

Microbial interactions, Food processing and Future foods

Advances in the methods for the inference of microbial associations in microbiomes

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Microbes rarely, if ever, live in isolation. As in other biomes, microbial interactions contribute to shaping the structure and dynamics of food microbial communities and eventually, to determining quality and safety.

Interaction between strains can be measured directly with cultivation-based methods which, in most cases, are low throughput. The availability of massive amounts of metataxonomic and metagenomic data allows the opportunity to infer microbial association networks (MAN) with co-occurrence and mutual exclusion relationships. Mining these networks provides data which can then be experimentally tested and are relevant for improving our understanding of food, host and environmental biomes, and the impact of disturbances such as disease, fermentation and spoilage on network structure and stability. A further level of complexity is captured by inter-domain MAN, a type of bipartite network which are meant to capture the relationships between bacteria and fungi, which shape the properties of fermented foods like sourdough products, cheeses, and many other. Ideally, it can also allow the identification of keystone taxa and of interactions which are critical for network structure, function and stability. This information can be used for the rational design of synthetic microbial communities and microbiome-based starters, which capture and reproduce the complexity of metabolic interactions which shape food properties in a superior way compared to the traditional starter systems.

In this talk I will briefly illustrate network science concepts and vocabulary as applied to microbial communities and provide an outline of available methods for the inference and analysis of MAN, with emphasis on usability and quality of the results.

I will then illustrate the advantages and pitfalls of this approach and provide a few examples of how large databases can be exploited to generate consensus bacterial and fungal microbial association networks of important fermented foods.

A multi-omics approach to unravel microbial and metabolic drivers of biogenic amine formation in traditional cheeses

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This study aimed at investigating the occurrence and drivers of biogenic amine (BA) accumulation in traditional cheese samples, grouped by milk origin and cheesemaking technology into Hard (HC), Pasta Filata (PF), Soft (SC), and Non-Bovine (NB) categories. Each cheese was characterized for physical and chemical features, microbial counts (culture-dependent), free amino acids (FAAs), and BAs. Distinct profiles emerged with regard to the BAs content observed in cheese categories, as NB cheeses exhibited the highest concentrations of tyramine, putrescine, and cadaverine, while histamine was more prevalent in bovine HC and PF cheeses. BA levels did not consistently increase with ripening time, and only weak correlations were found with precursor amino acids, proving how microbial activity and environmental conditions are key determinants in BA formation. Besides, culture-based analyses were complemented with a culture-independent multi-omics approach. Total DNA was extracted from each cheese and subjected to 16S rRNA gene amplification (metabarcoding), allowing taxonomic resolution of microbial communities at genus and, in many cases, species level. Based on the microbial community profiles, PICRUSt analysis (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States) was performed to predict the microbiota pathways specifically involved in amino acid decarboxylation and proteolysis. In parallel, gas chromatography-based metabolomics was carried out to evaluate the volatile profiles associated with different cheeses and microbial consortia. This integrated, multi-factorial strategy enabled the identification of specific patterns linking cheese microbiota, functional potential, and metabolic outputs. Preliminary findings suggest that certain microbial genera are associated with elevated BA concentrations and distinct proteolytic profiles. These results highlight the importance of combining classical and omics-based tools to better understand the complexity of microbial-driven processes in cheese ripening and safety. Future work will further explore these associations to develop predictive models for BA risk and improve process control in traditional cheese production.

Functional traits of *Propionibacterium freudenreichii* and its lactic acid bacteria consortia as starters for faba bean fermentation

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Propionibacterium freudenreichii, a secondary starter in cheese, is increasingly used in the fermentation of plant-based food due to its ability to produce the active form of vitamin B12. However, how *P. freudenreichii* responds and adapts to plant substrates remains unclear, which is critical for selecting proper starters for plant-based food fermentation. Furthermore, designing the fermentation process focusing on the interaction between starters, raw materials, and processing conditions is paramount to mitigate some of the nutritional challenges typical of plant materials such as faba beans.

In this study we evaluated the main phenotypic traits of 14 strains of *P. freudenreichii* derived from cheese and from culture collection (DSMZ) in standard medium (YEL). The best performing strains were cultivated in faba bean model medium (FBM) to define their metabolic switch using a phenotypic microarray by OmniLog for profiling carbon source utilization. Furthermore, consortia of PAB and lactic acid bacteria (LAB) selected based on use of RFOs, and having phytase, peptidase I, aminopeptidase N and beta- glucosidase activity were designed. Strains and consortia performance was assessed with kinetics of growth and acidification in FBM, and HPLC analysis for RFOs, acids and vitamin B12.

Strain-level differences were observed (peptidase activity and acids production) with certain strains e.g., AS9 and 282 better suited for fermentation of FBM. The carbon utilization patterns of the two strains had little overlap between FBM and YEL. The metabolic performance was lower over a wide range of carbon sources when grown in FBM compared to YEL. Nonetheless, vitamin B12 synthesis was significant in FBM. When in consortia with LAB, 282 was able to synthesize higher content of vitamin B12 than when cultivated alone (ca. 90 ng/mL vs 75, respectively) and RFOs were partly degraded. In conclusion, design of fermentation strategy allows achieving desired changes in the raw materials.

Promoting innovation of ferMENTed fOods (PIMENTO): the COST ACTION CA20128 outcomes

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Present in all European diets, fermented foods (FF) hold a strategic place due to the benefits they offer in terms of nutrition, sustainability, innovation, cultural heritage and consumer interest. The potential of FF for improving human health, but also driving food innovation and local production in the next decades, has become highly relevant. Here, we summarise the public repositories and publications where the results and outcomes of PIMENTO project, a COST Action CA20128 (Promoting Innovation of ferMENTed fOods), which started in November 2021 and will end in November 2025, are available. COST Action CA20128 is supported by COST (European Cooperation in Science and Technology). The project has been conceived to face the challenge of federating the scientific community and key stakeholders working on FF, with the aim of collectively advancing scientific evidence of their health benefits, building a benefits/risk approach in order to promote multi-modal innovation and respond to the expectations of European communities. The achieved results and outcomes supported the long-term goal of PIMENTO, which is to place Europe at the spearhead of innovation on microbial foods, promoting health, regional diversity, local production at different scales, contributing to economic and societal development as well as food sovereignty.

The project is the result of a collective contribution, summarising the efforts of the PIMENTO Community (PIMENTO Core Group and the PIMENTO Members). The author would like to acknowledge the support by COST (European Cooperation in Science and Technology; www.cost.eu), in the framework of COST Action CA20128 (Promoting Innovation of ferMENTed fOods; https://fermentedfoods.eu/).

Exploiting the potential of *Weissella* and *Periweissella* strains: new insights and novel applications in functional food design

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The genus *Weissella* and the recently described genus *Periweissella* are underutilized for their potential, and more knowledge on these strains is necessary. Six type strains of *Weissella* and *Periweissella* were characterized for their genetic determinants evaluating the carbohydrate metabolism pathways and the consumption of different carbon sources. The probiotic potential and safety assessment of the strains were performed with genomic and phenotypic characterization and the biotechnological characteristics evaluated. *Periweissella beninensis* LMG 25373T exhibited positive result in motility assay, which was also confirmed by Transmission Electron Microscopy. The high adhesion capability and resistance under simulated gastrointestinal conditions indicated significant probiotic potential. Moreover, it produced a dextran-type exopolysaccharide (EPS) with a distinctive high degree of branching (approximately 29 % α-(1 → 3)-linkages) compared to other EPS-producing lactic acid bacteria. Therefore, the application as starter for the preparation of plant based dairy alternative was evaluated. Growth performance, acidification, and proteolytic activity were investigated in various plant-based substrates (lentil, chickpea, and rice flours water soluble extracts (WSE) and compared to *Leuconostoc pseudomesenteroides* DSM 20193 (EPS-producing strain) and *Lacticaseibacillus rhamnosus* GG (probiotic strain). After the selection of the most promising fermentation conditions, *P. beninensis* LMG 25373T was used as starter in gelatinized and non-gelatinized legume-based substrates, achieving EPS synthesis levels of up to 1.3 g/100 g and 2.7 ± 0.2 g/100 g, respectively. A plant based gurt alternative prototype was developed, and fermentation with *P. beninensis* LMG 25373T resulted in a marked increase in viscosity, which remained stable during refrigerated storage. This suggested that the EPS distinctive structural characteristics contribute to its functionality as a hydrocolloid. Moreover, the strain maintained high viability throughout storage, a critical feature for its application in probiotic food products.

From north to south: microbial profiles and ecological characteristics of Italian fermented sausages

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Traditional Italian fermented sausages harbour a complex microbiome that significantly influence sensory properties and safety. In this study we collected 49 spontaneously fermented sausages across 15 different regions in Italy to investigate the resident microbiome in order to highlights the role of autochthonous microbes in shaping distinctive aroma and sensory profiles. Whole shotgun metagenomic sequencing, culturomics, metabolomic analysis and sensory acceptability were then performed.

The results revealed high heterogeneity in microbial and sensory profiles, even among samples from the same region, underlining the strong impact of recipes and processing. Samples clustered according to the strain level differences each characterized by one dominant taxon: *Pediococcus pentosaceus*, *Latilactobacillus curvatus*, *Staphylococcus xylosus*, and *Latilactobacillus sakei*, respectively. A comparative functional and genomic analysis further linked microbial taxa to specific sensory attributes, offering deeper insights into microbial contributions to product quality. Beyond microbial profiling, this work adopts an ecological perspective to explore the interplay between fermentation. The findings contribute to a better understanding of artisanal fermentation processes and support the preservation of local food heritage.

Synergistic application of microbial fermentation and High-Pressure Homogenization for the valorisation of brewer's spent grains into functional food ingredients

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Food waste continues to be a major global challenge. The Food and Agriculture Organisation (FAO) estimates that around a third of the food produced for human consumption is lost every year. This also includes large quantities of food by-products such as cereals, fruit and vegetables. In response, the PROACTIVE project (PRIN 2020, Prot. 2020CNRB84) aims to valorise food by-products through the production of bioactive peptides for the development of health-promoting food ingredients. Among the strategies investigated, microbial fermentation and high pressure homogenisation (HPH) have proven to be promising biotechnological tools to improve peptide release from substrates like brewer's spent grains (BSG), an important by-product of the brewing industry. In this framework in the present study, BSG were subjected to optimised fermentation with selected yeast and lactic acid bacteria strains, with and without prior HPH treatment (500–1000 bar). The resulting fermented products were analysed for their peptide content. The most promising samples were further characterised to evaluate their functional and safety properties. Microbial growth was assessed and cytotoxicity was analysed using MTT assays in human intestinal cell lines (Caco-2 and HT-29) to confirm the biocompatibility of the samples. In addition, the fermented extracts were tested for their protective effect against sodium dodecyl sulphate (SDS)-induced inflammatory stress in Caco-2 and HT-29 cells. Their anti-inflammatory effect was also validated in RAW264.7 macrophages by quantifying nitric oxide (NO) production. The results showed that the functional properties of the fermented products were influenced by both the microbial strains and the applied HPH treatment. Overall, these results emphasise the potential of BSG fermentation as a sustainable approach to produce peptide-rich food ingredients with health-promoting properties.

Another brick to understand the fructophilic beahviour of *Hanseniaspora valbyensis*

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Among non-conventional yeasts, *Hanseniaspora* is known for its high capacity to produce aroma compounds during fermentation and for its apparent reduced ability to completely utilise sugars in some ecosystems, due to sensitivity to ethanol or antimicrobials produced by other yeasts. Although the capacity of *Hansenisapora* species (e.g. *Hanseniaspora valbyensis*) to grow on fructose was previously reported, no fructose-specific transporters were identified. This study aimed to characterize fermentation behavior and scout potential fructose transporters of *H. valbyensis* biotypes, isolated from pomegranate. Well characterised strains of *Saccharomyces cerevisiae* and *Kluyveromyces marxianus* were used as references. The capacity to assimilate diverse sugars was first assessed using agar minimal medium (MM) supplemented (at 2%) with glucose, fructose, lactose, xylose, maltose, or sucrose. *H. valbyensis* grew at highest level on MM plates containing glucose or fructose. Starting from this result, growth, sugar consumption and metabolite production were investigated during liquid cultivation on glucose, fructose, glucose and fructose. Among the yeast species under investigation, *H. valbyensis* was the only one able to consume fructose and glucose after cultivation on MM broth, thus suggesting potential fructophilic behavior. Whole genome analysis was performed to identify potential sugar transporters of *H. valbyensis*, and a phylogenetic tree was built. The analysis showed that some sugar transporters of *H. valbyensis* were close to Ffz1 fructose facilitator of *Zygosaccharomyces bailii*. Ffz transporters are phylogenetically distinct from all the other previously characterized hexose transporters and more similar to drug transporters. Further research will focus on the role of this candidate Ffz1-like transporter in *H. valbyensis*.

Advancing "Ewiss" cheese production and strengthening its geographical identity through the use of selected autochthonous lactic and propionic acid bacteria

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In response to the growing demand for product differentiation in the global dairy market, Ewiss cheese, an aged sheep milk cheese, was recently developed to enrich the portfolio of ovine cheeses produced in Sicily region (southern Italy). However, the use of commercial starter cultures does not effectively establish a strong connection between the product and its geographical origin, nor does it contribute to a distinctive identity. To address this, the present study explores the development of "Natural Ewiss" cheese (N-EC), aiming to reinforce its territorial identity through the application of selected autochthonous lactic acid bacteria (LAB) and propionic acid bacteria (PAB) as starter cultures. LAB strains of *Streptococcus thermophilus* from the University of Palermo culture collection were used as natural whey starter cultures, while *Propioniciclava flava* was introduced separately once the milk reached pH 6.4. Microbiological monitoring from milk to ripened cheese revealed a dominance of thermophilic LAB after curdling and a significant increase in PAB populations after nine months of ripening. Metagenomic analysis confirmed *Streptococcus* and *Lactobacillus* as the predominant genera throughout the ripening period. The ripened cheeses showed a protein content of 28.19% (dry matter) and a favorable fatty acid profile, notably rich in oleic acid. Volatile compound analysis identified 31 compounds, with acids, particularly propionic acid, being the most abundant. Sensory evaluations indicated that cheeses produced with indigenous strains received higher scores than those made with commercial cultures. This study demonstrates that integrating Swiss cheese-making techniques with local microbial biodiversity can enhance both the sensory qualities and the territorial identity of Ewiss cheese, thereby contributing to the preservation of regional agri-food heritage and supporting the economic sustainability of the local dairy sector.

Phage-host coevolution: a strategy for improving lytic bacteriophage efficacy against STEC in food systems

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Shiga toxin-producing *Escherichia coli* (STEC) are major foodborne pathogens responsible for severe illnesses such as hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS). Transmission commonly occurs through undercooked meat, raw dairy products, and fresh vegetable products, with ruminants identified as the primary reservoirs (EFSA, 2020), posing an increasing threat to public health.

This study investigates the potential of bacteriophages as biocontrol agents, specifically examining the impact of coevolutionary training to enhance phage efficacy against bacterial resistance mechanisms. To achieve this, a lytic bacteriophage, originally isolated from breeding farms (Mangieri et al., 2020), was subjected to 25 sequential rounds of coevolution with diverse STEC strains, including both O157 and non- O157 serogroups, through overnight incubations.

Following this coevolutionary process, the "trained" bacteriophage was re-isolated and its complete genome was sequenced. A direct comparison of the genomes of the wild-type and trained phages was performed to identify genetic alterations responsible for enhanced efficacy. Furthermore, both the wild-type and trained bacteriophages were analyzed for their burst size, providing insights into their replication efficiency. The STEC strains that showed resistance to phages were also isolated and characterized to assess their mutations, offering a reciprocal view of the evolutionary arms race.

The trained phage consistently exhibited improved suppression of STEC growth in kinetics over 3 days compared to the wild-type. The trained phage significantly reduced STEC in challenge test on food matrix, demonstrating its biocontrol capabilities in a practical setting.

These comprehensive results underscore the phage coevolution as a proactive strategy to improve phage-based biocontrol of STEC in food applications, highlighting the possible role of evolutionary adaptation in enhancing phage therapeutic potential.

Exploring yeasts as novel natural biopreservatives: antibacterial potential against foodborne pathogens

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Foodborne bacterial illnesses pose a significant public health concern due to their high incidence, hospitalization rates and potential severity. Although chemical preservatives are widely used in the food industry to extend shelf life and limit microbial contamination, growing consumer concerns about their potential toxicity arisen. In addition, the detection of antimicrobial-resistant bacteria in food products, have sparked interest in alternative preservation methods such as biopreservation.

To date, the most common natural antimicrobials applied in food preservation originate from essential oils, plant extracts or bacterial metabolites. In contrast, the antibacterial activity of yeasts remains poorly investigated, despite their potential to produce bioactive compounds with inhibitory effects on pathogenic microorganisms.

This work focused on assessing the *in vitro* antibacterial activity of different yeast strains against major human pathogens, including *Escherichia coli, Staphylococcus aureus, and Listeria monocytogenes*. Initial tests, carried out using agar diffusion technique and plate-on-plate assay for production of inhibitory volatile organic compounds, showed that some yeast strains were able to significantly reduce bacterial growth.

Following these promising results, further trials were conducted on different food matrices such as meat and milk, in order to evaluate the performance of selected yeasts under applied conditions. Mechanisms underlying this antagonistic activity, as well as applications of biologically active yeasts in food sector, would be the final goal of this study. Indeed, the effective application of these yeast strains may be the use as natural biopreservatives to enhance food safety and extend product shelf life, offering a sustainable and consumer-friendly alternative to traditional chemical preservatives.

Variability of insect associate microbiome as function of rearing substrate and production scale: a case study on black soldier fly larvae reared on food-related side-streams

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Black soldier fly (*Hermetia illucens*) larvae (BSFL) can grow on organic side streams, including food waste. Insect microbiome mainly depends on the substrate used for their rearing. Therefore, this study aimed to evaluate how substrate and rearing scale affect the microbial community associated with the insect body.

Two experiments were conducted at lab and semi-industrial scale. BSFL were reared on six substrates obtained by combining several side-streams generated from food production. Microbial variability was measured in insects, substrate and frass.

Raw 16S sequencing data were filtered to remove microbial taxa occurring in less than 10% of samples and characterised by less than 1% of total abundance. The obtained dataset was rarefied to the lower read depth and analysed to determine alpha and beta diversity. Analysis of variance (ANOVA) run on observed diversity and Simpson index showed no statistically significant differences between larvae grown on different substrates ($p = 0.262$ for observed diversity; $p = 0.094$ for Simpson index), although significant differences were detected for the Shannon index ($p = 0.018$). PERMANOVA (permutations = 999) run on Beta diversity computed through the Bray-Curt distance metric displayed significant differences among samples ($p = 0.003$). Interestingly, microbial diversity in insects was always higher than in substrates and frass.

Venn diagrams constructed between substrate, frass and insects within the same treatment showed approximately 10% of shared bacterial families, with larvae characterised by the highest uniqueness. In contrast, frass was marked by highly shared genera. Insects from different treatments shared more than 30% of the microbiome, confirming, on the one hand, the presence of a core microbiome, while on the other hand, showing high variability as a function of the rearing substrate. Analysis carried out on a larger scale closely followed the results obtained at the lab scale, although higher variability was detected.

Antimicrobial and antibiofilm effects of nanoemulsions essential oils to control pathogen in cheese

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The use of nanoemulsions containing essential oils to control pathogens in cheese has garnered significant attention due to their enhanced antimicrobial and antibiofilm properties compared to traditional formulations. The encapsulation of essential oils within nanoemulsions improves their stability, bioavailability, and efficacy against foodborne pathogens, providing a promising strategy for food preservation. This study evaluated the antibacterial and antibiofilm effects of nanoemulsions incorporating *Thymus vulgaris* (TV), *Thymus capitatus* (TC), and *Origanum hirtum* (OH) essential oils against *Listeria monocytogenes*, Shiga toxin-producing *Escherichia coli*, *Staphylococcus aureus, Enterococcus faecium,* and *Salmonella Typhimurium* on cheese. Minimum Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentrations (MBC) were determined using a microtiter plate assay. Additionally, the influence of subinhibitory concentrations of the nanoemulsions on biofilm formation was assessed in 96-well plates.

Cheese surfaces were then inoculated with pathogens and treated individually with subinhibitory concentrations of the nanoemulsions, supplemented with nisin at concentration allowed by the EU regulation. Non-inoculated and untreated samples served as controls. All experiments were conducted in triplicate. Samples were analyzed immediately after the inoculation and after 22 days of storage.

All essential oil nanoemulsions exhibited antibacterial and anti-biofilm activity against all pathogens. TV and TC showed a broader and more consistent effect, while OH was particularly effective against Salmonella and E. coli. The addition of nisin in nanoemulsion enhanced the biofilm-inhibiting properties, with TV and TC combined with nisin achieving a 60% reduction in biofilm formation. Results from cheesemaking trial confirmed a statistically significant reduction (2-3.5 Log CFU/g) of viable count of all pathogens on treated samples compared to controls.

These findings highlight the potential of essential oil-based nanoemulsions, particularly when combined with nisin, as effective natural alternatives for improving the microbial safety of cheese.

Microbiome mapping in the meat food chain: from farm to fork

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Meat spoilage is driven by microbial activity, and it poses significant economic and health challenges. This longitudinal study characterizes the microbiome taxonomic and functional profiles along the 4 beef processing chains, from farm to fork, across two seasons to understand the dynamics of microbial contamination and antimicrobial resistance genes (ARGs) along the beef chain. We collected 712 samples per two seasons, covering 4 animals per facility, contact and non-contact surfaces in different locations (farm, slaughter hall, chiller hall, boning hall, retail packing hall, and carcasses, processing end meat, and matured meat). We carried out shotgun metagenome sequencing using Illumina NovaSeq. The raw sequences were filtered using prinseq-lite, and assembled contigs were produced using megahit. Taxonomic and functional profiles were generated using Kraken2 and Phabox2 for bacteriophages, while Card, BacMet, VFDB, and Mobile Genetic Element Finder tools identified ARGs, biocide resistance genes, virulence factors, and MGEs. Statistical analysis and visualization were performed in R. Alpha diversity indices (Shannon, Simpson, and Richness) showed no significant differences across facilities or production phases. While the alpha-diversity was significantly higher in winter samples, it was compared to the summer samples and among different among the major locations. In beta diversity, farm samples had distinct microbiomes compared to other stages, with overlaps observed between all major locations. Nine consistent bacterial strains, 30 ARGs, and 21 biocide resistance genes were widespread and strongly linked with integrons, transposons, and plasmids, indicating ARG dissemination. This study highlights microbiome dynamics and ARG dissemination in the beef production chain, guiding targeted interventions to enhance food safety and reduce ARG spread.

Whole-genome sequencing of a vineyard-related *S. kudriavzevii* strain reveals links to the hybrid population with *S. cerevisiae*

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Saccharomyces kudriavzevii is a cryotolerant yeast of increasing interest in wine fermentation due to its low ethanol yield and high glycerol production, that could find interesting applications in reducing wine ethanol content. Despite these desirable traits, there is only a single documented case of *S. kudriavzevii* being isolated from grape musts to date, whereas its natural hybrids with *Saccharomyces cerevisiae* are more frequently encountered in wine-related environments, even though their origins remain unclear.

In this work, we report the first whole-genome sequencing of a vineyard-related *S. kudriavzevii* strain, isolated from fermenting grape juice in Italy. We performed a SNP-based population genomic analysis including both *S. kudriavzevii* and *S. cerevisiae* × *S. kudriavzevii* hybrids from various environments and regions. Our findings reveal that the hybrid strains form a genetically distinct population more closely related to the European lineage of *S. kudriavzevii* than to its Asian counterparts. Notably, the Italian strain shares some allelic variants at frequencies similar to those observed in hybrids, suggesting that the hybrid lineage may have originated from *S. kudriavzevii* parental strains closely related to the one we identified. Ongoing work aims to further characterize the physiological and fermentative properties of this newly isolated strain, which may provide insights into yeast evolution and support novel industrial applications.

Almond skin upcycling through LAB fermentation: technological and nutritional impacts in food productions

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Almond skin is a by-product of almond processing that is primarily used in animal feeding or fuel production. The first aim was the selection of starter cultures to be used in innovative food through a spontaneous fermentation of almond skin, and daily propagation based on Type I sourdough. LAB strains were isolated from fermented almond skin and evaluated for their EPS-producing activity. A selected pool of LAB strains was employed in a 24-hour fermentation of almond skin, to assess its efficacy at industrial by- product chain level. Results demonstrated that almond skin fermentation led to an increase in antioxidant and prebiotic compounds. In evaluating resilience to almond skin niche, two LAB strains (*Leuconostoc mesenteroides* UNLB14 and *Lactiplantibacillus plantarum* UNLB7) were selected for a stronger acidification power. At the same, these LAB enhanced the nutritional profile and contributed to the bioactive potential of the fermented almond skin. Furthermore, these strains showed an important rheological power supported by metabolomics profiles that highlighted metabolites conferring almond and floral notes.

The second aim of this study was evaluating the influence of the addition of fermented almond skin (FAS) and deoiled almond (DA) in gluten-free muffins formulation, focusing on their biochemical, functional, technological, and sensory effects. Muffins enriched with FAS exhibited significantly increased antioxidant activity and free amino acid content, particularly glutamate, alanine, and proline. The incorporation of FAS and DA also led to a significant reduction in the hydrolysis index (HI) and predicted glycemic index (pGI). Technological analysis showed modifications in crumb and crust colorimetric parameters and texture profile, while sensory evaluation revealed intensified aromatic notes and enhanced textural attributes in fortified formulations. VOCs analysis showed significant compositional shifts linked to FAS and DA enrichment.

These findings underscore the potential of upcycled almond by-products to enhance the nutraceutical, glycemic, and sensory profile of GF-bakery products.

Disruption of the gut microbiota composition of *Lethrinus mahsena* fish caused by helminth parasitic infection

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Fish Microbiota refers to the community of microorganism, including bacteria, fungi, viruses, and protozoa that live in the gastrointestinal tract. Most of this microorganism is bacteria especially, anaerobic bacteria, which plays a crucial role in the heath of the fish. Lethrinus mahsena, a species found throughout tropical and marine setting, is important to coastal ecosystems and has a high economic value. Parasitic infections in marine fishes become a significant public health concern worldwide. There are several diseases, which can cause by parasites; for instance, narcosis, inflammation, and blood vessels congestion especially in the gut of fish, which may may affect the economic importance. However, little is known about the impact of parasitic infections on the composition of fish microbiota. The aim of this study was to examine the bacteria composition of fish that infected by different type of parasites, such as Cestoda, Trematoda, Nematoda. 24 of cecum of *Lethrinus mahsena* fish were isolated and 16srRNA was sequenced by Illumina MiSeq. Analyses were carried out using QIIME-2. In this study, we determined five phyla, which are Firmicutes, Proteobacteria, Fusobacteriota, Actionbacteriota, and Bacteriodota. We found that infected by Trematoda caused a decline of the Vibrionaceae, which had member of facultative anaerobes and were capable of fermentation foods. However, the member of Enterobacteriaceae was increased, which had many of the more familiar pathogens, such as, Escherichia coli, Klebsiella, and Shigella. Also, we found that infected by Trematoda and Nematoda increased the member of the *Shewanellaceae*, which reported had members of seafood spoilage bacteria. Also, we found that the composition of *Lachnospiraceae* was decreased, which had members that ferment diverse plant polysaccharides to produce short-chain fatty acids. We concluded that Trematoda were the most effect on the gut microbiota of fish, compared to Cestoda and Nematoda because Trematoda settled in the hindgut of fish where the microbiota lives there. Cestoda and Nematoda are mostly found in the midgut of fish. Thus, this study highlights the fish gut microbiota alterations that could change the physiology of fish, which become a primary source of foodborne infections.

In-depth microbial analysis of Sant'Agostino table olives flavored with wild fennel (*Foeniculum vulgare* Mill., 1768)

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This study explores the microbial ecology of Sant'Agostino green table olives, a traditional Apulian product flavored with wild fennel (*Foeniculum vulgare*), using a polyphasic approach that integrates culture-dependent and culture-independent methods. The olives underwent spontaneous fermentation, and both fruit and brine samples were analyzed to characterize cultivable and non-cultivable microbial populations. Microbial enumeration revealed lactic acid bacteria (LAB) and yeasts as the dominant groups. LAB levels reached 7.3 Log CFU/g in fruit and 6.4 Log CFU/mL in brine, while yeast populations were higher in brine (5.6–5.9 Log CFU/mL) than in fruit (4.5–4.9 Log CFU/g). *Enterococcus faecium* and *Candida tropicalis* emerged as the most prevalent species. The absence of Enterobacteriaceae, coliforms, *Salmonella* spp., and *Shigella* spp. confirmed the microbiological safety of the product. Spore-forming bacteria, such as *Bacillus velezensis* and *Priestia endophytica*, were detected exclusively in brine at approximately 2.5 Log CFU/mL. Culture-based analysis identified six LAB species, including *Lactiplantibacillus plantarum*, *Lactiplantibacillus pentosus*, and *Leuconostoc mesenteroides* subsp. *cremoris*. High-throughput sequencing of the 16S rRNA gene (V3–V4 region) confirmed the dominance of *Enterococcus* (up to 81.7% relative abundance in brine) and *Leuconostoc* (up to 40.3% in fruit), with *Lactobacillus* present at lower levels (<1%). Alphaproteobacteria were notably abundant in fruit samples, reaching up to 90.1% in one batch. The integrated culture-dependent and culture-independent approach provided a comprehensive overview of microbial diversity, highlighting the potential of selected LAB and yeast strains for enhancing the quality of traditional fermented products. Further studies are needed to specifically assess the technological and functional properties of these strains, with the aim of improving the quality and health-promoting features of Sant'Agostino table olives.

Harnessing safe bacteriocin-producing enterococci isolated form plants for bio-protection and for reducing anti-nutrients & indigestible oligosaccharides in soymilk yoghurt analogues

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Plant-based yogurt analogues have gained significant popularity recently. Key driving factors are lactose intolerance and the perception that plant-based foods are more sustainable than the dairy alternative. When preparing plant-based yoghurt analogues, often conventional yoghurt cultures are used, despite the fact that plant-based substrates differ significantly from milk. We have explored whether three safe *Enterococcus* strains can be used for fermenting soy milk and found that all isolates could acidify soymilk to a pH below 4.7 using an initial inoculum of 10^6 cells/ml (standard inoculum when preparing yoghurt). *Enterococcus* is renowned for producing antimicrobial peptides, or bacteriocins. Thus, we investigated whether the four strains could confer a bioprotective effect and a strong antimicrobial effect was observed against tested pathogens. Soymilk contains the three indigestible and flatulence inducing α-galactosides raffinose, stachyose, and verbascose, which are undesirable carbohydrates in soy milk. Three strains demonstrated α-galactosidase activity. Additionally, all tested strains had an ability to degrade phytic acid, an unwanted anti-nutrient found in many plant-based foods, including soybeans and soy milk. Overall, our findings indicate that enterococci have great potential for use in plant-based fermentations where they contribute to enhancing nutrient availability.

Evaluation of the impact of milk thermization on the microbiological, physico-chemical, and fermentative characteristics in the production of Fontina PDO

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This study investigated the effect of thermization of raw milk (64°C for 40 s) on the main microbiological, fermentative, and physico-chemical parameters of Fontina PDO cheese. The aim was to verify the compatibility of this treatment with the PDO production standards, evaluating its influence on cheesemaking and the final profile of the ripened cheese. The experimental design included the comparative production of cheese wheels from raw and thermized milk, with analyses conducted on both milk and cheese at different stages of ripening. The fermentation process was monitored by evaluating pH, time to acidification onset, and pH drop rate. Results showed a similar behavior between the two groups, with a slightly faster acidification kinetics in the thermized milk samples, likely due to reduced microbial competition in favor of the added starter cultures. From a microbiological perspective, thermization significantly reduced the load of undesirable microorganisms, particularly *Escherichia coli* and coliform bacteria, thereby improving the hygienic quality of the milk. However, a marked decrease in the biodiversity of the native lactic microflora was observed, especially among thermophilic lactobacilli, necessitating the use of authorized starter cultures to ensure proper fermentation.

Analyses of ripened cheese confirmed that levels of lactococci and thermophilic streptococci remained adequate during maturation, whereas lactobacilli counts were consistently lower in cheese made from thermized milk. Populations of *Leuconostoc spp.*, yeasts, and coagulase-positive staphylococci were found to be similar in both groups. Physico-chemical analyses did not reveal significant differences between the two types of cheese.

In summary, milk thermization appears to be an effective technology for improving microbiological safety in the production of Fontina PDO, while maintaining the sensory and quality standards required by the PDO specification. However, it requires the integration of starter cultures to compensate for the loss of native microbial biodiversity and to preserve cheesemaking quality.

Valorization of chickpea flour with selected microbial strains to improve its nutritional, functional and gut health-promoting properties

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As global demand for sustainable and functional protein sources grows, legumes such as chickpeas (*Cicer arietinum* L.) have gained interest for their rich protein content and bioactive compounds. However, their nutritional potential is limited by the presence of antinutritional factors, including raffinose-family oligosaccharides (RFOs) and aldehydes, which also contribute to undesired sensory characteristics. This study investigated the targeted fermentation of chickpea flour using microbial strains selected for their ability to reduce undesirable compounds and the growth of spoilage microorganisms and pathogens, and to improve chemical-physical and sensory attributes. *Lactiplantibacillus plantarum* LP23, either as a single strain or in co-culture with *Debaryomyces hansenii* Y15A, was applied to enhance nutritional and functional profile of chickpea flour and assess its impact on gut health-promoting properties. Fermented samples were characterized for their peptide and amino acid content, starch digestibility, antioxidant activity, and volatile aroma profile. Fermentation led to a significant increase in soluble peptides and total amino acids content, a reduction in damaged starch, and enhanced antioxidant capacity. A significant decrease in total aldehydes was also observed, improving the sensory profile. The fermented ingredients and their application in pasta formulations (30% fortification) were subjected to standardized gastrointestinal digestion (INFOGEST protocol) and assessed using a 24 h *in vitro* gut fermentation model. The digested samples showed a beneficial modulation of gut microbial composition, characterized by increased production of short-chain fatty acids (SCFAs), particularly acetate. These results demonstrated that strain-specific fermentation of chickpea flour can effectively improve its nutritional and functional properties while promoting a gut microbiota profile associated with host health, offering a promising strategy for developing functional plant- based ingredients for sustainable nutrition and gut health.

Microbiological and biochemical profiling of sourdoughs for sweet leavened baked goods application

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Sourdough is a mixture of flour and water spontaneously fermented by lactic acid bacteria (LAB) and yeasts with leavening and acidifying capacities used to produce leavened baked goods. The microbial species in sourdough play a crucial role in the development of flavor, texture, nutritional availability and shelf life of baked goods.

This study explores three different production protocols for the utilization of sourdough to produce Pandoro, an Italian traditional sweet leavened baked good, coming from three different industrial production sites at three different stages of their protocol. A combination of culture-dependent and culture independent methods (e.g., shotgun metagenomics), as well as biochemical characterization were used to identify the sourdough's microbial composition, create a biobank of sourdough strains and explore its stability-resilience after fermentation based on its production protocol and biochemical markers. To improve stability-resilience of resulting sourdoughs, diverse fermentation protocols will be tested altering temperature and time.

From each sourdough at in the different stages of the fermentation protocol, have been isolated twenty yeasts, and sixty presumptive LAB in aerobic and anaerobic conditions. Preliminary results showed variation in terms of microbial cell density along the sourdough fermentation protocol, considering LAB/yeasts ratio and biochemical parameters. Therefore, future research will focus on the management of the fermentation protocol to improve sourdough stability. The outcomes of this study will support the development of customized sourdough protocols for leavened sweet baked goods.

Onion extract rich in organosulfur compounds as a clean-label preservative for ready-to-eat hummus

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In recent years, consumer demand for ready-to-eat (RTE) foods has significantly increased. Among these, Mediterranean-origin products such as hummus have gained widespread popularity. This study evaluated the antimicrobial effect of a standardized onion (*Allium cepa*) extract containing 8% organosulfur compounds (mainly thiosulfinates and related derivatives) as a natural preservative for RTE-hummus. Firstly, the *in vitro* antimicrobial activity was assessed against key food-borne pathogens and spoilage microorganisms, including *Escherichia coli*, *Listeria monocytogenes*, yeasts and filamentous fungi. The results demonstrated that the onion extract exhibited significant antimicrobial activity. To validate this antimicrobial potential, the extract was added as an ingredient in hummus, to assess its impact on product shelf-life and quality during storage at 4°C for 28 days. In addition, a challenge test was performed using *Zygosaccharomyces bailii*, *L. monocytogenes* and *E. coli* inoculated at an initial level of 4 log CFU/g. A positive control sample was inoculated with each microorganism without the use of onion extract and a reference sample was treated with a traditional preservative (potassium sorbate). The findings in the food model confirmed the strong *in vitro* antimicrobial activity, demonstrating that the extracts effectively delayed spoilage, especially yeast proliferation, contributing to shelf-life extension. The extracts significantly reduced *Z. bailii* populations compared to the inoculated control, outperforming the effect of potassium sorbate. The onion extract was also able to control both *L. monocytogenes* and *E. coli*. After 28 days of refrigerated storage, microbial reductions up to 4 log CFU/g compared to the corresponding positive controls were shown. These findings highlight the potential use of onion-derived organosulfur compounds as clean-label preservatives in hummus, with an organoleptic impact fully compatible with the product's aroma profile.

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A preliminary investigation on lipid accumulation by *Metschnikowia pulcherrima*

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In the context of circular economy, microbial biomasses are promising alternative source for food and feed production, and oleaginous yeasts, producing intracellular lipids for over 20% of dry cell weight, can be key resources. *Metschnikowia pulcherrima* is a non-conventional yeast increasingly explored for wine-related applications, and it has an industrial potential as an oleaginous species, being able to grow on different agro- food residues, tolerating low pH and temperature. This study aimed to investigate qualitative methods suitable for high-throughput screening of a large collection of *M. pulcherrima* strains for lipids accumulation. Ten strains of *M. pulcherrima* clade were used as test collection. Strains were grown at lab scale in a high C/N medium, and lipid droplets were observed under the microscope. The qualitative plate method with Rhodamine B dye was evaluated in both visible and UV light, then compared with lipid extraction and gravimetric quantification. While qualitative protocols did not robustly reveal the oleaginous behaviour, quantitative data showed that one novel strain, VUCC500, could be an interesting single-cell-oil strain, with glucose as a carbon source. Remarkably, its yield increased in preliminary tests with alternative low-cost carbon sources. Further studies are necessary to define reliable protocols for primary screening of *M. pulcherrima* strains in VUCC collection, with VUCC500 as positive control. However, since VUCC500 genome is sequenced, further analyses will be focused on this strain, targeting safety aspects necessary for this non-QPS species.

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Microbial milk-clotting enzymes used in raw-milk hard cheese production do not influence natural whey starter and ripened cheese microbiota

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During cheesemaking, milk proteins are coagulated to curd causing a phase transition from liquid to gel. The process can be rennet- and/or acid-induced and consist of casein denaturation followed by their aggregation. Rennet has been traditionally produced from animals or plants. However, the worldwide increasing cheese consumption, associated with cheese producers' interest in opening their market to consumers with restrictive dietary preferences, has led to alternative milk-clotting enzymes, such as microbial coagulants. However, a recognized drawback of inconstant cheese quality is mainly related to the low specificity of the proteolytic activity of these coagulants. In addition, although microbial coagulants have been extensively correlated with chemical-physical and technological aspects, their effects on cheese microbiota have been poorly investigated. Here, we show how the microbial community of natural whey starter (NWS) and raw-milk ripened cheese (RRC) produced using three microbial coagulants from *Rhyzomucor miehei* do not substantially differ from controls produced using calf rennet. While the use of one microbial coagulant reduced the NWS acidifying performance, albeit with no consequences on cheese production, none of the other conditions tested revealed a significant effect on the phenotypic traits, acidification time and rate of the NWS microbiota. In accordance, experimental and control NWS samples were found to be similar in species richness and relative abundances, as confirmed by non-significant alpha and beta diversity indexes. No differences in the taxonomic composition were found between the experimental and control cheeses ripened for 9 and 12 months.

Preliminary results demonstrate how microbial milk-clotting enzymes do not influence the cheese microbial community in the tested condition. However, only correlations with ongoing functional shotgun metagenomics, as well as chemical-physical and sensory analyses will confirm whether the use of microbial coagulants significantly alters the characteristics and the aroma of RRC produced using NWS.

Artichoke powder from wastes as a prebiotic-like compound for food formulations

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Artichoke residues (*Cynara scolymus L*) and by-products represent a valuable resource for composting, biochar production, or extraction of bioactive compounds for agricultural and industrial applications. Their sustainable management contributes to reducing environmental impact and enhancing the value of the artichoke supply chain.
In this research, artichoke powder, produced from by-products and waste, was used as an ingredient to produce fermented milk containing probiotic microorganisms. First, the target strains (*Lactobacillus acidophilus* La5, *Lactiplantibacillus plantarum* c19, *Bifidobacterium animalis* subsp. lactis DSM 10140, *Bifidobacterium longum* subsp. *infantis* DSM 20088, *Lactiplantibacillus plantarum* DSM 1055) were inoculated in a saline solution (0.9% NaCl), containing different amounts of artichoke (0.05–0.5% w/v) to evaluate dose-dependent effects on viability over 72 hours. In a second step, the impact of environmental stressors, such as pH (4.5, 6.5, 8.5) and temperature (15, 37, 45°C), was assessed through a full factorial design, both in presence and absence of 0.3% artichoke extract. Results indicated that the powder modulated microbial survival in a strain-specific and condition-dependent manner, with notable protective effects at suboptimal pH or elevated temperatures.
An *in vivo* application involved the development of a functional dairy beverage. UHT whole milk was supplemented with 0.3% artichoke extract and inoculated with *Lpb. plantarum* c19 and *L. acidophilus* La5. Fermentation proceeded until target pH values were reached, followed by refrigerated storage. Microbial viability, pH stability, and chemical parameters (fat, protein, lactose, amino acids and fatty acids) were monitored over 28 days. Artichoke powder supported probiotic persistence and acidification kinetics, suggesting its suitability as a prebiotic additive in synbiotic formulations. These findings highlight the technological and functional versatility of artichoke by-products in probiotic food systems.

Integrated microbial and chemical screening of plant-based products: a nanopore and UHPLC-MS/MS approach: a step toward safer future foods

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Ensuring the safety of plant-based food products requires advanced tools for detecting both microbial and chemical hazards. In this study, we present an integrated dual-approach workflow targeting biological and chemical contaminants in a diverse array of plant-derived products. From a microbial perspective, we implemented a rapid, portable sequencing strategy for the detection of Bacillus spp. based on Oxford Nanopore MinION and tuf gene amplicon sequencing. The approach enabled species-level identification of heat-resistant spores in 72 commercial products. *Bacillus* spp. were detected in 87.5% of samples, with the *B. cereus* group found in 17.5%, demonstrating the method's superior sensitivity compared to 16S rRNA- based sequencing and culture-based analysis. Concurrently, to address chemical safety, a UHPLC-QTOF-MS screening was conducted to detect pesticide residues and transformation products across the same product set. Using a non-targeted, data-independent acquisition (DIA-AIF) method and advanced spectral library matching (including custom and open-source databases), a wide variety of suspect compounds were identified. Specifically, residues such as Lobendazole, Metamitron-desamino, Demethylisoproturon, Imiprothrin, and Prodiamine were recurrent across multiple food categories including beverages, meat analogues, and supplements. In total, more than 70 distinct contaminants, including herbicides, fungicides, veterinary drugs, and natural alkaloids (e.g., senecionine), were tentatively annotated, often co-occurring in single samples. The co-detection of chemical residues and microbial risks in the same products (e.g., beverages and meat analogues) emphasizes the necessity of integrated, high-throughput screening systems. The presented dual workflow combines sensitive chemical profiling with real-time, species-specific microbial identification, offering a comprehensive solution for food quality monitoring. This methodology is particularly suited for modern food safety applications where rapid turnaround and detailed resolution are critical for risk assessment and consumer protection.

Selection of lactic acid bacteria able to produce conjugated linoleic acid

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Consumer awareness of the correlation between a balanced diet and human health has generated a growing demand for functional foods. Conjugated linoleic acid (CLA), particularly the isomers cis9, trans11 and trans10, cis12, has attracted considerable interest. Milk, dairy products, and ruminant meat are rich in CLA, which has demonstrated numerous health benefits, such as immune system modulation, body weight management, antidiabetic effects, and antihypertensive properties. Certain microorganisms, such as lactic acid bacteria (LABs), are able to convert linoleic acid, contained in food matrices, into CLA.

The purpose of the present study was to select CLA-producing LAB strains to formulate a starter culture suitable for cheesemaking. A total of 34 LABs were screened for their ability to produce CLA. In detail, 0.5 mg/ml of pure linoleic acid was added to each strain. After incubation at 30°C for 48 hours, fatty acids were extracted by employing 2-propanol and n-hexane and read spectrophotometrically at 233 nm. The strains were subsequently screened for technological properties, including coagulation ability, acidifying activity, and survival at different salt concentrations.

Out of the 34 tested strains, seven showed the ability to produce CLA. Among these, two strains, ascribed to the *Lacticaseibacillus rhamnosus* and *Lactiplantibacillus plantarum* species, able to release 10.74 μg/ml and 10.85 μg/ml of CLA, respectively, were selected as promising CLA-producers starters. In conclusion, the selected strains could be suitable to produce a functional cheese.

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Application of ozone to enhance the safety of lupin beans

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Recent interest in plant-based proteins has led to an increased focus on lupin-based products. However, safety concerns are related to the potential presence of *Diaporthe toxica* and quinolizidine alkaloids (QAs) in lupin. Although limited information exists on this topic, *Diaporthe toxica* is the primary producer of the mycotoxin phomopsin A (PHO-A), while QAs cause heart disorders. To enhance food safety, ozone technology well fits, having demonstrated significant potential in the inactivation of both microorganisms and mycotoxins in other food matrices, though no evidence is available about PHO-A and QAs.
This study aimed to assessing the possibility of using ozone to reduce the concentration of PHO-A and QAs, enhancing the safety of lupin beans, but first it was necessary to characterize *Diaporthe toxica* and its metabolites production.
Various substrates were inoculated using mycelial plugs and incubated at 25 °C for 21 days to evaluate *Diaporthe toxica* growth and metabolic activity. Subsequently, lupin beans were inoculated similarly and exposed to 7 ppm aqueous and gaseous ozone for 4, 6 or 8 hours. PHO-A and QAs concentrations were determined by SPE extraction followed by UHPLC-MS/MS analysis, while fungal load was investigated by microbiological sampling.
Results showed the ability of the mould to develop mycelium on different substrates, while spores production only occurred in some cases. PHO-A production demonstrated a diverse fungal adaptation to various environments, reaching values up to 1082 ppm. Concerning ozone, aqueous treatments provided more effective results than gaseous ones in reducing PHO-A by roughly 20%, while different patterns were identified for QAs treatment.
In conclusion, findings reveal that ozone treatments break new ground in using non-thermal technologies for food safety, particularly regarding lupins, as ozone can be applied in the washing phases of the process. Moreover, aspects of *Diaporthe toxica* were deepen, thus upgrading the knowledge about this fungal species.

Enhancing the microbiological, physicochemical, and sensory properties of fresh ovine 'Tuma' cheese through the addition of feeding prickly pear by-product silage in the ewe's diet

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This study investigated the potential of prickly pear by-product silage as feed in the diet of lactating ewes on improving the quality characteristics of fresh sheep cheese "Tuma". Tuma cheese is a fresh pressed Sicilian cheese made from ewe's milk obtained by pressed cheese technology. A Latin square design was used to feed three different groups of ewes: a control diet (CTR) consisting of hay and concentrate, a diet supplemented with prickly pear peels silage (PPP), and a diet containing prickly pear pulp, peels and seeds silage (PPS). Milk and cheeses samples were evaluated for their microbiological, physicochemical and sensory properties. The total phenol content (TPC), antioxidant capacity (AOC) and fatty acid profiles of cheese were investigated. The microbiological profile of milk and cheese was similar among all diets, indicating no negative effect of PPP and PPS on fermentation process. MiSeq Illumina analysis identified high concentrations of Lactic Acid Bacteria (LAB) in almost all cheese samples. The physical-chemical analysis not showed significant differences between the cheeses. The highest TPC levels of cheese were observed in PPP, followed by PPS, while CTR presented the lowest levels ($P < 0.01$). CTR cheese showed a significantly ($P<0.01$) higher saturated fatty acids (SFA) % than PPP and PPS. Moreover, poly unsaturated fatty acids (PUFA) % was significantly lower in CTR than PPP and PPS cheese. These results suggest that PPP and PPS silage can be partially incorporated into the diets of dairy ewes because it tends to improve the microbiological, physical-chemical and sensory characteristics of fresh "Tuma" cheese.

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Italian fermented foods with a status geographical indication: wine, cheese, bread, semi-dry sausage, and table olive

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Italy has a significant gastronomic heritage with several Geographical Indications. Many of these products are fermented, including cheese, bread, table olive, semi-dry sausage, and wine, representing the diversity of traditional Italian foods transformed through a desired development of yeasts, bacteria and filamentous fungi in the raw matrix. Here, we propose an overview of the variability of these foods and beverages in terms of categories and geographical distribution, providing information on the associated biodiversity, the technological regime used and the management of microbial resources. This diversity is observed through the scientific literature, proposing a picture of research activities that promote innovation in this sector, in line with trends oriented towards improving the quality and safety of production. Selecting tailored case studies, a special discussion is dedicated to the selections and characterisations of bacteria, yeasts and filamentous fungi associated with these productions and to the emerging trends that combine the valorisation of microbes/microbiomes with the protection and promotion of traditional, typical and artisanal fermented foods.

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Ecology in a vessel: material-driven microbial dynamics and their influence on wine fermentation outcomes

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The fermentation vessel material significantly influences the microbial ecology, chemical composition, and sensory profile of wine. Stainless steel, oak, and clay each shape the fermentation environment differently, due to their specific physical and chemical properties, such as porosity, oxygen permeability, and interaction with the wine matrix.

Building on this framework, the study explores how fermentation vessel material influences microbial ecology and wine development throughout vinification, also evaluating the impact on chemical-physical parameters and sensory profiles of the final wines.

Three common oenological vessels: stainless steel (inert, impermeable), oak barrique (porous, rich in extractables), and terracotta amphora (micro-oxygenating, thermally insulating) were investigated. Friulano grapes were chosen for their regional relevance, microbial dynamics were monitored at key vinification stages and the wines were characterised for their chemical-physical and sensory properties.

The results clearly show that the fermentation vessel material significantly influences the microbial ecosystem, due to intrinsic properties like porosity, oxygen permeability, and potential for microbial colonization. While winemaking practices also affect microbial succession, especially early on, the material itself emerged as the main factor. Clay-fermented wine displayed the highest microbial complexity, with more non-Saccharomyces yeasts and both lactic and acetic acid bacteria, likely due to clay's porous, breathable nature. Voltametric, total polyphenols, and catechins clearly differentiated the clay wine from those in stainless steel and oak, confirming the measurable impact of vessel material on wine composition and sensory profile. Overall, the vessel acts not just as a container, but as an active modulator of fermentation dynamics and final wine quality.

Transcriptomic and metabolic responses of *Listeria monocytogenes* to enterocin B: insights from a cheese-based model system

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In our previous work we succeed in identifying and characterizing an enterocin B from *Enterococcus faecium* RM12 isolated from raw milk. In the present development this study we aimed at investigating the transcriptomic response of *Listeria monocytogenes* when exposed to this enterocin in a model cheese environment. A cheese model broth based on lyophilized Brie cheese was used to mimic realistic conditions and evaluate Listeria gene modulation in response to Enterocin B both as a purified postbiotic and in co- culture with live *E. faecium* cells.

For this purpose, two complementary transcriptomic approaches were employed. In the first, *L. monocytogenes* was exposed to *E. faecium* cell-free supernatant (CFS) containing the enterocin B. The expression of selected genes associated with stress response, virulence, motility, and adhesion was monitored through RT-qPCR using the 2^−ΔΔCt method. In the second strategy, *E. faecium* RM12 and *L. monocytogenes* were co-cultured under the same cheese-based conditions, and RNA-seq analysis was performed to highlight global transcriptomic changes in both organisms. All experiments were conducted at 7 °C and 37 °C, with sampling at 3 and 18 hours to assess temperature- and time-dependent effects.

This dual approach offered deeper insights into *L. monocytogenes* adaptation strategies in response to both postbiotic and live-cell antagonism. Preliminary findings reveal clear transcriptional modulation, particularly in genes related to environmental stress and motility, while expression of virulence-associated genes remained generally low. Additionally, gas chromatography was used to assess metabolic shifts, suggesting potential links between transcriptomic changes and the production of specific metabolites.

Overall, this study demonstrates the strength of an integrated transcriptomic and metabolomic approach for unraveling microbial interactions and stress responses. Moreover, it emphasizes the added value of using a cheese-based model system that closely mimics real food matrices, thereby enhancing the ecological and technological relevance of the results.

Exploitation of yeast fermentation to convert citrus by-products into a functional ingredient for beverages

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Citrus processing residues account for over 70% of the total fruit weight. To valorise these by-products, microbial fermentation offers a promising strategy to induce chemical and physical modifications that enhance the bioavailability of residual nutritional and nutraceutical compounds, thus enabling their reuse in food sector. This study focuses on the development of an orange-carrot-lemon beverage enriched with a fermented clementine peel as an innovative functional ingredient, obtained using a selected strain of *Saccharomyces cerevisiae*.

Yeast selection involved 27 strains of *Saccharomyces* and non-*Saccharomyces* species and was based on their ability to ferment the residue in terms of cell viability, growth rate, modification of the volatile sensory profile, and increase in phenolic content. A *S. cerevisiae* strain was ultimately selected for its greater performances. The functional ingredient was produced by fermenting clementine peel for two days, followed by freeze-drying. It was then added to the beverage at different concentrations (1, 1.5, 2, and 3% w/v). The effects of ingredient addition were assessed in terms of changes in colour, volatile profile, phenolic and β-carotene content, antioxidant activity, and sensory attributes.

Results showed a significant increase ($p < 0.05$) in total phenolics and β-carotene contents following the ingredient addition. Furthermore, the aroma profile was enhanced due to high levels of terpenes such as 3- carene and bergamotene, resulting from the metabolic activity of the selected yeast strain. Sensory panel evaluations highlighted improved pleasantness and aromatic complexity in the ingredient-added beverages compared to the control one. In conclusion, this research significantly contributes to the circular bioeconomy by offering an effective strategy for valorising agro-food by-products. The findings pave the way for innovative, healthy, and distinctive food products, supporting industry demands and global sustainability goals.

Recovery of raw milk microbial biodiversity for the development of safe, biodiverse natural starter cultures in dairy production

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The preservation of indigenous microbial biodiversity in raw milk is essential for maintaining the authenticity, safety, and sensory complexity of traditional dairy products, especially Protected Designation of Origin (PDO) cheeses. The widespread use of commercial starter cultures, typically consisting of a limited selection of lactic acid bacteria (LAB), has improved fermentation reliability and product safety. However, this practice has significantly reduced microbial diversity in both dairy environments and final products, threatening the unique sensory profiles and regional identities of traditional cheeses. In this study, a freeze-dried natural starter culture (NSC) was developed directly from raw ewe's milk, deliberately avoiding thermal decontamination or selective enrichment to preserve the native microbiota. The NSC was further supplemented with highly diverse, strain-characterized mixtures of *Streptococcus thermophilus* and *Lactobacillus delbrueckii*, both isolated from indigenous sources. Molecular and phenotypic analyses confirmed that the NSC maintained high species and strain-level diversity, including both starter and non- starter LAB. Safety assessments, including screening for antibiotic resistance and virulence genes, verified the absence of pathogenic or spoilage organisms, even among non-QPS species. The technological performance of the starter culture was validated in both artisanal and pilot-scale dairy plants under varying conditions. Across all trials, the starter demonstrated robust acidification, consistent curd formation, and stable cheese yields. Microbial analyses confirmed the successful establishment and persistence of a diverse LAB community throughout milk, curd, and ripened cheeses, resulting in products with stable physicochemical properties and distinctive sensory characteristics. Consumer acceptance studies indicated no sensory decline in cheeses aged up to 180 days. These findings demonstrate that freeze-dried, raw-milk- derived starter culture can effectively preserve microbial biodiversity and cultural heritage while ensuring safety and technological reliability, offering a sustainable alternative to commercial starters and supporting the quality and typicity of PDO cheeses.

Valorisation of brewer's spent yeast: impact of pre-treatment and autolysis on mannoproteins, β-glucans, and bioactive compounds for food packaging applications

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The brewing industry generates large quantities of by-products, including brewer's spent yeast (BSY), which represents a particularly valuable matrix due to its high content of bioactive compounds such as β-glucans, mannoproteins, and fatty acids. However, the valorisation of BSY requires strategies that can promote the release of these components, which are often trapped within the cell wall. In this study, ten samples, one control and 9 samples derived from different BSY pre-treatment and autolysis processes, were analysed. The samples were fractionated into three components: autolysate (LIS), protein hydrolysate (PH), and yeast cell debris (YCD). The aim was to evaluate the impact of these treatments on the release of bioactive compounds. Parameters such as pH, dry matter content, β-glucan and mannoprotein levels, lipid profile, antioxidant activity, and antimicrobial activity were analysed.

The results showed that autolysis treatments (24 and 72 hours) significantly promoted the release of β-glucans and mannoproteins, with higher concentrations of these compounds found in the LIS fractions. Lipid analysis identified palmitic acid (C16:0) as the most abundant fatty acid, followed by oleic acid (C18:1), with a notable presence of medium-chain fatty acids (C8:0–C12:0) in LIS and YCD. All samples exhibited measurable antioxidant activity, although no clear trend was observed among the different fractions, likely due to treatment variability. Antimicrobial assays revealed selective inhibitory effects against *Listeria monocytogenes*, particularly in YCD fractions at both 5% and 10% concentrations, suggesting a correlation with the presence of short-chain fatty acids, as well as other bioactive compounds such as polyphenols and glutathione. These findings highlight the potential of BSY as a source of bioactive compounds for food applications, including natural antimicrobials and antioxidants, and for the development of sustainable packaging materials in line with circular economy principles.

Microbial load and antibacterial activity shift in hydra upon immune challenge with LPS

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The immune system is a fundamental feature of animal life, deeply rooted in evolutionary history. It has evolved under intense selective pressure and serves as the foundation of animal defense against pathogens. At the same time, animals cannot be viewed as single, independent organisms; rather, they are holobionts—complex ecological units composed of the host and its associated microorganisms. As a cohesive biological entity, the microbiome exerts significant influence on the host's fitness, development, behavior, physiological functions, and immunity. To investigate how immune challenges affect the host and its interaction with microbiota, lipopolysaccharide (LPS) was used. However, the inconsistencies observed in LPS-induced responses across different invertebrate species highlight the need for further research. In this study, we aimed to understand how early-diverging animals like Cnidarians recognize and respond to LPS, and how these responses influence their symbiotic microbial communities. We used Hydra as an animal model. To assess changes in immune response, we exposed Hydra to two concentrations of LPS and tested the antibacterial activity of peptides extracted post-exposure against *Pseudomonas* sp. Furthermore, to evaluate the consequences of immune activation on microbiota, we measured the cultivable bacterial load in Hydra following LPS treatment. The study revealed a clear trend: both low and high LPS treatments resulted in significantly different inhibition zones compared to the control group. In addition, LPS exposure also led to a significant difference in cultivable bacterial load relative to control, suggesting that immune activation impacts microbial abundance. These findings highlight the importance of immune challenges in modulating both host immune responses and host–microbiota interactions in early-diverging animals.

Advances in microbiome cryopreservation of apulian table olives

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Microorganisms and their associated microbiomes are fundamental to Earth's ecosystems, serving as primary drivers of essential biological processes. In the food ecosystem, microbial communities from fermented foods contribute not only to food safety and preservation but also to health and wellness benefits. In this context, Microbial Culture Collections represent strategic resources for ensuring food quality and supporting the development of nature-inspired bio-based fermented products. The increasing demand for sustainable food systems is encouraging both industry and research to explore the potential of complex microbial communities in food fermentation. However, a major bottleneck in the exploitation of microbiomes lies in the lack of standardized and effective protocols for their long-term preservation, as well as in the evaluation of preservation outcomes in terms of microbial viability and functional integrity. Recent researches within the ongoing EU project SUS-MIRRI.IT (N° IR0000005) have provided new insights into best practices for preserving, reviving, and reusing microbiomes from various sources, including fermented foods. This study presents original data on the cryopreservation of microbiomes associated with olives and brines, assessing the effectiveness of protocols involving two storage temperatures and the use of glycerol and dimethyl sulfoxide (DMSO) as cryoprotectants. The microbial consortium was studied before and during a 1-year storage period using a culture-dependent approach, RNA- based metabarcoding analysis, and metabolic profiling with the Biolog Ecoplate. The results showed that after 1 year of cryopreservation, the viability of the microbial consortium slightly decreased, regardless of the cryoprotectant used, and no significant changes in the metabolic profile were observed. Moreover, the metabarcoding analysis showed no significant differences in relative abundances after the storage period. This integrated approach can be extended to other fermented foods, contributing to a deeper understanding of microbial ecology and reinforcing the pivotal role of culture collections in maintaining viable and functional microbiomes for future applications.

Portraying table olives microbial communities: a full characterization via metagenomic analysis

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Fermentation is among the earliest and most cost-effective techniques for food production and preservation. Beyond its economic and historical significance, fermentation naturally contributes to reducing the bulk of materials for easier transportation, eliminating harmful substances, improving the nutritional quality and visual appeal of foods, decreasing cooking energy demands, and enhancing overall food safety. Due to these characteristics, fermented foods (FFs) have always been an interesting field to be explored.

The aim of this project is to exploit the table-olive microbiome to identify the main strengths and weaknesses that can be improved during each step of the production process.

More than 300 samples were collected from different Mediterranean countries - including Italy, Spain and Greece - with particular emphasis on the Oliva di Gaeta PDO variety. In total, 800 bacterial and yeast colonies were isolated. Shotgun metagenomic analysis was then performed to characterize the microbiome associated with different cultivars.

Results show that *Lactiplantibacillus pentosus* and *Lactiplantibacillus plantarum* are the predominant lactic acid bacteria species. Yeasts such as *Pichia membranifaciens*, *Candida boidinii* and *Wickerhamomyces anomalus*, closely associated with product spoilage, were also found. Among the regions studied, the natural table-olive microbiome from Greece displayed the highest variability.

These findings improve our understanding of the factors that influence final product quality and may help stakeholders identify microbial markers for olive varieties.

Microbiota characterization and *in vitro* assessment of the anti- inflammatory activity of Kefir

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Kefir is a widely recognized health-promoting fermented beverage, produced through the symbiotic activity of lactic acid bacteria (LAB), acetic acid bacteria (AAB) and yeasts. This study aimed at characterizing the microbial kefir's community and to assess its potential anti-inflammatory properties at the intestinal level. To this end, a comparison was made between a commercially kefir and a *lab-scale* artisanal kefir, including its associated grains. To quantify the microbial populations, both culture-dependent and culture-independent methods (qPCR) were employed. Following microbial profiling, the study further explored the potential anti- inflammatory effects of the kefir samples by evaluating the expression of inflammation-related genes in an inflamed intestinal epithelial cell model (NCM460). Specifically, the expression levels of cytokines involved in inflammatory bowel disease (IBD), namely IL-6, IL-10, IL-12, IL-17A, and IL-23, were measured after exposure to the different kefir samples, subjected to a simulated *in vitro* digestion (INFOGEST). Microbial analysis revealed a higher abundance of LAB and yeasts in both the artisanal kefir beverage and its grains, compared to commercial kefir. Moreover, other bacteria such as *Leuconostoc* spp. and *Acetobacter* spp., as well as the yeasts *Pichia kluyveri* and *Saccharomyces* spp. were detected, underscoring the microbial diversity of these samples. Interestingly, the artisanal kefir beverage and its associated grains exhibited a greater ability to reduce the expression of pro-inflammatory cytokines compared to commercial kefir, showing a significant anti-inflammatory potential. Overall, these findings support the hypothesis that kefir's microbial composition contributes to its anti-inflammatory effects on the intestinal epithelium. The observed anti-inflammatory activity highlights the need for further research into the specific microorganisms and metabolites responsible for this bioactivity, which could ultimately guide the development of targeted functional food formulations aimed at gut health and inflammation management.

Microbial processes to valorise brewer's spent grain for innovative ingredients and healthy foods

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Current linear models in food production need to evolve towards more sustainable, circular economic systems. The brewing industry serves as a case study in this context. Brewer's spent grain (BSG), the principal by-product of the brewing process that still contains valuable nutrients, is frequently subjected to inefficient and energy-intensive disposal methods, such as drying or animal feed. Optimizing BSG management could enhance resource efficiency, mitigate environmental impact, and generate economic benefits for brewers.

This project aims to evaluate different approaches to convert BSG into valuable food ingredients, with a focus on sustainability and resource efficiency. Firstly, a low-energy drying process will be developed to preserve the nutritional properties of BSG, resulting in a stable powder suitable as a food ingredient.

Furthermore, microbial bioprocesses will be optimized to extract and valorize the bioactive components of BSG. Specifically, microbial consortia, which include yeasts and lactic acid bacteria, will be selected as starters for bakery products. Filamentous fungi will be screened to produce microbial proteins from BSG through optimized fermentation. Finally, a high-fiber bread, incorporating BSG and supplemented with probiotic bacterial spores, will be developed, with the objective of creating a "postbiotic" product. For all proposed approaches, comprehensive chemical and microbiological analyses will be carried out to evaluate the composition of BSG and its derivatives, focusing on the quantification of fibers, proteins, lipids, phenolic compounds, and mycotoxins. Sensory evaluations will also be implemented to assess the acceptability of the BSG-derived innovative products.

Brewers' spent grains as reservoir of yeasts for applications in brewery

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Brewers' spent grains (BSG) are the solid and insoluble fraction of malted barley remaining after the mashing and filtration phase of the brewing process. Due to their high moisture and fermentable sugar contents, BSG represent a perishable material, suitable for microbial development. There is limited information regarding the BSG occurring microbiota; hence, the study aimed to investigate the BSG microbiota and evaluate their potential as a reservoir of yeasts for brewing. The BSG (from 8 artisanal breweries) were kept in plastic buckets, left open for 48 h in the brewery environments. Yeast and lactic acid bacteria (LAB) populations occurring in the BSG were quantified and identified. Yeasts were detected in 7 samples, ranging from 2 log CFU/g to 5 log CFU/g; LAB ranged from 4 log CFU/g to 9 log CFU/g. 9 LAB species were identified, *Pediococcus pentosaceus* was found in 4 out of 8 samples. 11 yeast species were identified in the BSG samples, *Maudiozyma humilis* and *Pichia fermentans* were detected in 4 out of 8 breweries. The *S. cerevisiae* isolates were subjected to inter-Δ PCR typing to compare the profiles with those of the commercial *S. cerevisiae* strains used in the breweries, highlighting the presence of indigenous strains. Yeasts were tested for β-glucosidases activity and growth in beer wort. The β-glucosidases activity was variable and generally higher in the non-*Saccharomyces* strains; on the contrary, they showed lower growth in wort compared to *S. cerevisiae*. The *S. cerevisiae* indigenous strains were tested for small–scale brewing, showing results comparable to a commercial strain in terms of ethanol production, sugar consumption and growth rate. The findings highlighted BSG as a valuable and rich source of yeasts to be potentially suitable for application in beer production as starter cultures (*S. cerevisiae* strains) or as potential co-starters (the non- *Saccharomyces* strains).

Development and shelf-life assessment of a fruit-flavoured fermented legume-based dessert

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In recent years, growing consumer interest in sustainable and functional plant-based foods has driven research into dairy alternatives, with a particular focus on fermented legume-based matrices. In this context, a new fruit-flavoured fermented dessert was developed using a fava bean (*Vicia faba*)-derived base. The goal was to create a high-protein product with a gel-like consistency and potentially postbiotic compounds.

The base matrix was obtained by fermenting fava bean with commercial starter cultures (*Lactobacillus delbrueckii subsp. bulgaricus* and *Streptococcus thermophilus*). A lipid blend of shea butter and sunflower oil was added to the formulation. After fermentation and acidification, the mixture was combined with fruit purée, natural flavourings, and dietary fibers. The final product was packaged in 125 g containers, sealed, and treated with High Hydrostatic Pressure (6000 bar for 120 seconds), then stored at 8°C. A 120-day shelf- life study was conducted to monitor microbiological and biochemical stability, including the analysis of soluble proteins, peptides, and volatile compounds.

HHP treatment resulted in a 5-log reduction in lactic acid bacteria, which continued to decline during storage, reaching levels below 3 log CFU/g after 80 days. *Bacillus spp.* resulted the predominant spoiling group. Although it was below 1.0 log CFU/g after HHP, after 20 days it reached about 4.0 log CFU/g.

However, this level has not changed throughout the rest of the shelf-life.

The pH of the product, initially 3.71, gradually increased up to 4.20 by day 80. This slight rise did not compromise microbiological safety or product quality. Volatile compound analysis showed a terpene- dominated profile due to fruit flavourings added in the formulation. During shelf-life only minor changes of the volatile profiles were observed.

In conclusion, the HHP treated fava bean-based fermented dessert resulted microbiological and biochemical stable for over 120 days, supporting its potential as a functional, plant-based alternative.

Microbial dynamics and functionalities of synthetic microbial communities for biocellulose production

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Bacterial cellulose (BC) is a biopolymer used for several applications in food and biomedical fields. In the Project SynBioCell, 12 synthetic microbial communities (SMC) were constructed by assembling acetic acid bacteria (4 AAB, core strains), lactic acid bacteria (3 LAB, helper) and yeasts (3 Y, helper), which naturally cooperate in some fermented foods (e.g. kombucha, kefir), to boost BC production, overcoming the constraints related to individual strain metabolism, and ensuring metabolic diversity and cooperative interactions. The best (SMC2, SMC9, SMC11) and worst (SMC7) communities were select to evaluate the microbial dynamics and cell compartmentalisation (liquid fraction, liquid/BC interface, BC layer) during BC production (plate counting, BC yield, 1H-NMR spectroscopy). Moreover, SMC2 (Y-free consortium) was differently re-combined to investigate the effect of LAB on BC production (plate counting, 16S rRNA-based qPCR, BC yield, 1H-NMR spectroscopy). The relative expression (RT-qPCR) of cellulose synthase complex (CS) was also evaluated. SMC2 led the highest BC yield, compared to the single AAB strains. LAB were able to grow in HS and move to BC layer, while Y developed to a lesser extent in all compartments. The homofermentative and heterofermentative metabolism of LAB differently affected the BC yield, although lactate accumulation seems to have a stimulatory effect. Excess ethanol in Y-containing communities led high acetate concentrations (due to its oxidation by AAB) impairing BC yield. qPCR confirmed the overexpression of CS genes in LAB-containing consortia. Our results showed that targeted SMCs could be efficiently used as BC-production machinery, and provided further insight on metabolic networks and dynamics of microbial consortia. The metabolic abilities of selected SMC will be also exploit for degradation of more complex substrate (e.g. agri-food wastes) to promote a sustainable end cost-effective BC production.

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Turning by-products into bioactive compounds: solid-state fermentation of tomato waste for GABA production by *Levilactobacillus brevis* Y1

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The agri-food industry faces the dual challenge of minimizing the environmental impact of waste disposal while effectively valorizing these by-products through the extraction of health-promoting nutritional compounds. Among the available biotechnological strategies, microbial fermentation offers a sustainable and efficient approach to convert such residues into high-value compounds. Tomato pomace (TP), an abundant by-product of the agri-food industry, is particularly rich in glutamic acid (L-Glu), the precursor of the bioactive compound γ-aminobutyric acid (GABA). Several lactic acid bacteria can synthesize GABA from L-Glu trough the glutamate decarboxylase (GAD) system. Therefore, *Levilactobacillus brevis* Y1, a known GABA-producing specie, was selected to ferment the TP under Solid-state Fermentation (SsF) conditions to address sustainability concerns and reduce water consumption. The study aimed to investigate the key parameters - glutamic acid content, pH, temperature, and time - that regulate the GABA production to maximize the fermentation yield using the Central Composite Design (CCD) - Response Surface Methodology (RSM). The L-Glu conversion into GABA was assessed trough chromatographic technique (HPLC). The fermentation product derived from the optimized conditions was subsequently analyzed for its polyphenol content and the presence of potentially bioactive peptides. *L. brevis* Y1 TP fermentation resulted in efficient GABA production, reaching approximately 20 mg/g of TP. Moreover, the model generated through the RSM approach demonstrated high significance and strong predictive capability. Finally, the TP was considered a suitable growth matrix for the tested strain, prompting further investigation into its prebiotic potential. Overall, TP fermentation by *L. brevis* Y1 represents a promising strategy for the sustainable production of bioactive compounds from agri-food waste with potential applications in the food and nutraceutical sectors.

Monitoring the bacterial community of spontaneous fermentation of *Tenebrio molitor* powder by using culture-dependent and culture-independent methods.

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Tenebrio molitor (yellow mealworm) is notable for its nutritional profile, which is characterised by high biological value proteins, unsaturated fatty acids, and microelements. Concurrently, its characteristics provide favourable conditions for microbial survival and growth. This work explored the microbiota that developed during the spontaneous fermentation of a dough made from *T. molitor* powder and the creation of a fermented *T. molitor* ingredient. *T. molitor* doughs were backslopped 10 times, and bacterial communities were evaluated using viable plate counts and the identification of lactic acid bacteria (LAB) through PCR-ARDRA, as well as by targeted metagenomic sequencing of the V3-V4 region of the 16S rRNA gene. LAB concentrations in the doughs began at 10^2 CFU/g, then rapidly increased to approximately 10^9 CFU/g, remaining stable until the end. The identification of LAB via the culture-dependent method revealed greater biodiversity in the initial backsloppings, with a gradual decline during the refreshments. Ultimately, only *Lactiplantibacillus plantarum* and *Levilactobacillus brevis* were detected. In contrast, the targeted metagenomic analysis showed an increase in the relative abundance of LAB towards the final backsloppings, where 10 LAB genera were found, including *Lactiplantibacillus* and *Levilactobacillus*. This increase corresponded to a reduction in unclassified bacteria; alpha diversity did not experience significant changes during the successive backsloppings. Notably, there was a significant presence of bacteria belonging to the *genus Kurthia*, known to produce chitinolytic enzymes. 8 LAB strains, isolated from the last back-slopping, were tested for their acidification and growth capacity in *T. molitor* powder for ingredient production. The fermented and dried ingredients exhibited a decrease in the *Enterobacteriaceae* population. This research enhances our understanding of bacterial dynamics in these specific matrices and provides a preliminary assessment of their potential for food applications.

Metschnikowia pulcherrima as biocontrol agent in the production of Vin Santo

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Bioprotection is an alternative strategy to chemical fungicides and bactericides to prevent the growth of spoilage microorganisms. Several studies demonstrated that *Metschnikowia pulcherrima* can inhibit the growth of undesirable yeasts in winemaking, without compromising the fermentation capabilities of *Saccharomyces cerevisiae*. A recent investigation at laboratory scale also proved that *M. pulcherrima* is effective in protecting grapes against fungal infections during withering in sweet wine (*passito*) production. Therefore, this study aimed to test the bioprotective capacity of some *M. pulcherrima* strains in real conditions of Vin Santo production, a Tuscan *passito* wine. The strains used in the experiment were selected from a *M. pulcherrima* indigenous strains collection isolated from social wasps found in vineyards of various DOCG areas in Tuscany. Using indigenous yeasts for bioprotection could avoid introducing exogenous microorganisms into the environment, allowing for a greater chance of success because these yeasts should have a higher fitness advantage than commercial strains. Thirty strains were tested to evaluate their antimicrobial properties against moulds and non-*Saccharomyces* yeasts, and three were selected. With the selected strains, a liquid preparation was created and dispersed on a portion of the grapes vertically hung for drying in the *vinsantaia*, the traditional ventilated room used in Vin Santo production. The microbial ecology and the chemical characteristics of grapes treated and untreated with the bioprotective preparation were described during 72 days of withering. The treated grapes showed the presence of two inoculated *M. pulcherrima* strains at concentrations of 5 log CFU/g throughout the withering period. The untreated grapes showed a mould presence 2-3 log units higher than the treated grapes, while the concentration of non- *Saccharomyces* and acetic bacteria was not significantly affected. The two strains were also found in the early fermentation phases, demonstrating a good ability to persist in the Vin Santo production process.

Enhancement of cultivation efficiency of environmental microorganisms using microfluidic droplet technology

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Most microorganisms remain uncultured (85–99%), limiting our understanding of microbial ecosystems and restricting access to potentially valuable biological resources. To enhance the cultivation efficiency of environmental microorganisms, we have focused on surfactant-stabilized microfluidic droplet techniques.

The droplets, suspended in an oil phase and ranging in volume from nanoliters to picoliters, can be generated at high speed—thousands per second—and stored by the millions in 1.5 mL tubes, enabling a space-efficient and high-throughput culturing method. Using this approach, various antibiotic-producing bacteria and previously unrecognized antibiotic-resistant bacteria have been isolated. In addition, previous studies have shown that microorganisms are confined within droplets, whereas certain molecules can diffuse between them. Enhancing microbial interactions mediated by the exchange of signaling molecules between droplets is expected to enable the cultivation of previously unculturable microorganisms.

In this study, we aimed to enhance the cultivation efficiency of environmental microorganisms using the high-density droplet cultivation (HDDC) method with reduced water-in-oil droplet size. Microorganisms from freshwater samples were encapsulated in either small or large droplets and cultivated in parallel.

Microorganisms cultured in droplets exhibited higher cultivation efficiency compared to conventional plate- based cultures. Notably, the cultivation efficiency in small droplets (27% to 48%) using the HDDC method was significantly higher than in large droplets (5% to 23%). We further observed that microbial community richness in droplets was higher than that in bulk cultures. Intriguingly, the HDDC method promoted the enrichment of *Candidatus* Nanopelagicaceae (acI lineage), a rarely cultivated bacterial group found in diverse freshwater environments. These findings demonstrate that the HDDC method, with reduced droplet size, offers a promising approach for improving microbial cultivation and expanding access to uncultured microbial diversity.

Submerged cultivation of *Laetiporus sulphureus*: growth profiling, co-cultivation screening, and prospects for side-stream valorization

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Laetiporus sulphureus is an edible, wood-decaying fungus with the ability to degrade lignocellulosic material, making it a promising candidate for the bioconversion of agro-industrial side-streams. To explore its potential for submerged fermentation, we first characterized the growth of two wild-type Danish isolates under defined conditions. The effects of carbon and nitrogen sources, initial pH, and temperature on fungal biomass production were systematically assessed.

These results provide a foundation for selecting agro-industrial side-streams with similar nutrient profiles as sustainable substrates for fungal cultivation. A range of Danish and Mexican side-streams is currently being tested for their suitability in submerged fermentation and for food-relevant biomass production.

Building on the observed impact of initial pH on fungal growth, we are now exploring co-cultivation with selected food-grade bacterial strains to enhance fermentation performance. These co-cultures aim to improve fungal biomass yield and metabolite production through synergistic microbial interactions.

Together, this work supports the development of *L. sulphureus* as a microbial platform for alternative protein production by integrating physiological characterization, substrate valorization, and microbial community design.

The effect of sub-MIC concentrations of stevia on biofilm formation and mature biofilm of *Staphylococcus aureus* isolated from dairy cattle

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Bacterial biofilm presents a significant clinical challenge in both human and veterinary medicine. This complex structure not only protects bacteria from the host immune system but also confers resistance to antibiotic treatment. As such, the search for substances capable of inhibiting biofilm formation or disrupting mature biofilms remains a key area of scientific interest worldwide.

Stevia (*Stevia rebaudiana*), increasingly used in the food industry as a natural sweetener alternative to sugar, has recently attracted attention for its potential antibacterial properties. In this context, the aim of our study was to evaluate whether stevia exhibits antibiofilm activity against field strains of *Staphylococcus aureus* (n= 30) isolated from dairy cattle.

In the first phase of our research, the minimum inhibitory concentration (MIC) of stevia was determined in accordance with CLSI guidelines. Subsequently, we assessed the impact of stevia at concentrations of 12%, 6%, and 3% on both biofilm formation and established (48- and 72-hour) mature biofilms. Biofilm quantification was performed using crystal violet staining in 96-well microtiter plates.

Based on the initial results, the most promising strains were selected for further analysis, including gene expression profiling of *icaD* and *eno*, as well as fluorescence and scanning electron microscopy.

Our findings suggest that stevia can inhibit biofilm formation by *S. aureus* strains to a certain extent; however, its effectiveness against already-formed, mature biofilms is considerably lower. Nevertheless, the data indicate that stevia interacts with biofilm-related mechanisms in these bacteria in diverse ways.

In our opinion, further studies are warranted, particularly those exploring combinations of stevia with other natural substances possessing antibiofilm potential.

Valorization of volatile fatty acids-enriched cheese whey by *Yarrowia lipolytica* for single cell oil production

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Food waste and by-products are often valorized through methane production; however, volatile fatty acids (VFAs) from acidogenic digestion can serve as renewable carbon sources for single cell oil (SCO) production. *Yarrowia lipolytica*, an oleaginous yeast with Qualified Presumption of Safety (QPS) status, can accumulate SCOs up to 20% of cell dry weight (CDW) on sugars and even more on hydrophobic carbon sources. This study, performed within the European project ONE EARTH, investigates SCO production by *Y. lipolytica* grown on cheese whey, a common dairy by-product, enriched in VFAs via acetogenic fermentation. Ten wild-type strains were screened on minimal medium with 0.2% acetic acid; seven showed good performance. Their growth was monitored for 72 hours across acetic acid concentrations from 0.2% to 1.0%. Strain-specific tolerance was observed. Based on their performance, three strains (Y3, RO3., RO2) were selected and tested on a VFA-enriched medium at 20°C and 5°C for 13 and 20 days, respectively. While growth was slower at 5°C, biomass was higher (Y3: 817.9 mg/100 mL), whereas lipid accumulation increased at 20°C (Y3: 79.8 mg/100 mL, 10% CDW). Fatty acid analysis showed C18:1, C18:2, and C18:3 accounted for 38–46% of total lipids. RO3. had the highest unsaturation index; Y3 yielded the most long- chain unsaturated fatty acids. Y3 was then grown on VFAs, obtained from squacquerone whey, and incubated at 20°C under static and dynamic conditions. Aeration did not enhance fat content (max 13% CDW), while static conditions increased SCO content (mainly composed of C18:1, C18:2, and C18:3, >50%) to 42% after 13 days incubation. These results support *Y. lipolytica* as a promising biocatalyst to convert dairy waste into valuable lipids for circular economy applications.

Closing the loop in dairy processing: fermented second cheese whey as a functional ingredient for breadmaking

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Due to its high organic load, second cheese whey (SCW), a nutrient-rich by-product of whey cheese making process, represents both an environmental challenge and a valuable opportunity for the dairy sector. Previous results have shown that SCW qualifies as a cost effective substrate for microbial growth and biobased productions. However, despite the numerous potential applications, interest in its exploitation and valorisation remains surprisingly limited. We have shown that *Propionibacterium freudenreichii* strains, selected for physiological, technological, and functional traits, can effectively transform SCW into a pro-bioactive preparation enriched in vitamins B9 and B12, and other bioactive metabolites. Based on these findings and considering the protein and mineral salt (particularly calcium and magnesium) content of ovine SCW, as well as the production of short chain fatty acids, and B vitamin vitamers by selected propionibacteria, this study explored the use of fermented SCW as an ingredient in breadmaking, with the aim of promoting circularity in the dairy sector. For SCW fermentation, a low-input, simple process was developed. The fermented SCW was then incorporated into bread dough, partially (50%) or fully (100%) replacing water. Results indicate a dose-dependent effect of SCW on dough viscoelastic properties. Moreover, fermented SCW improved the microbiological stability of the final bread, delaying mold growth and reducing mesophilic plate counts, thus highlighting its potential as a natural preservative. This approach offers a promising strategy for SCW valorization and supports sustainable practices in the dairy sector, creating new economic opportunities, particularly in marginal areas with limited economic resources and propensity to innovation.

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The postbiotic potential of microalgae

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In recent years, increasing attention has focused on food sources that support human health, particularly microorganisms and their bioactive compounds. One emerging concept is postbiotics, which the International Scientific Association for Probiotics and Prebiotics (ISAPP) defines as preparations of inanimate microorganisms and/or their components that confer health benefits on the host. Microalgae and cyanobacteria are photosynthetic organisms widely recognized for their high nutritional value and abundance of bioactive compounds, including antioxidants, polysaccharides, fatty acids, and pigments such as chlorophylls, carotenoids, and phycobiliproteins. These compounds persist even after cell inactivation and may contribute to a range of health benefits when consumed. Unlike probiotics, which must remain alive to be effective, microalgae are typically consumed as inactivated whole cells, aligning with the postbiotic definition. Evidence from studies, particularly those focusing on *Arthrospira platensis* and *Chlorella vulgaris*, supports their potential to act as health-promoting agents in the human diet. Furthermore, several species of microalgae and cyanobacteria are already approved for use as food and dietary supplements in many countries. Their inclusion in the postbiotic category could offer a novel approach to developing functional foods. However, the composition and bioactivity of microalgal products are significantly influenced by the specific strain used and by environmental growth factors such as light intensity, temperature, and nutrient availability. To better understand and harness the postbiotic potential of microalgae, it is essential that both researchers and producers clearly identify the strains used and provide detailed documentation of cultivation conditions. This work aims to evaluate the current scientific evidence supporting the classification of microalgae as postbiotics. It also discusses the importance of standardized preparation methods, regulatory considerations, and future directions for clinical validation. Expanding the postbiotic framework to include microalgae could significantly broaden the scope of functional nutrition and promote the development of novel health-promoting food products.

Lactic acid bacteria: safe and effective microorganisms for stabilizing food by-products for feed applications

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Food loss and waste (FLW) are major global challenges with significant economic and environmental consequences, primarily due to the costs and pollution associated with disposal methods such as landfilling. Approximately one-third of all food produced is lost or wasted across the supply chain. While composting and landfilling are conventional FLW management strategies, these methods contribute to greenhouse gas emissions and ecological damage. A promising alternative is the valorization of food by-products as feed for insect larvae, particularly black soldier fly larvae (*Hermetia illucens* L.), which can convert organic waste into valuable biomass. However, a key challenge associated with food by-products is their high perishability. They are prone to rapid spoilage due to contamination by undesirable microorganisms, leading to a loss of nutritional value. To address this, a microbial starter culture based on lactic acid bacteria (LAB) was developed to ferment food by-products and preserve their quality for use as larval feed. LAB strains from the UNIMORE Microbial Culture Collection (UMCC) were selected for their ability to produce organic acids and antimicrobial compounds (i.e. bacteriocins). Antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli* was assessed using agar spot tests and broth microdilution assays on cell-free supernatants (CFS), with pH-neutralized CFS used to separate the effects of organic acids from other inhibitory compounds. Two seasonal feed formulations (simulating winter and summer diets) were created using food waste and inoculated with the LAB starter. These were fermented at 27 ±3°C for 48 hours and then fed to the larvae. Behavioral and morphological analyses were conducted to assess palatability and growth performance. This study presents a sustainable approach to FLW management by stabilizing food waste through fermentation and repurposing it as insect feed. The method reduces reliance on landfills and supports scalable insect production, contributing to circular economy objectives.

Whey enhancement by targeted fermentation: increased bioaccessibility of amino acids and BCAAs

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AURA project aims to develop functional foods for over-75 population, using whey as a nutritionally promising ingredient to counteract sarcopenia. The study evaluated the increase in branched-chain amino acids (BCAAs) and free amino acids through fermentation of sweet whey with previously selected proteolytic strains.

Whey fermentation was tested with two different strains of lactic acid bacteria (LAB), namely: *Lactiplantibacillus plantarum* E676 at 37°C and *Lactobacillus helveticus* 1386 at 42°C together with their combination at 37°C (COMB). Analyses were performed at different fermentation times (T0h, T12h, T15h, T18h, T24h). Both optical density (OD) measurements via spectrophotometer and plate counts were conducted. Unfermented whey was analysed using the Bradford assay for total protein quantification.

Fermented samples were then *in vitro* digested using the INFOGEST system to assess digestibility. Pre- and post-digestion fermented samples underwent ninhydrin assay for total amino acids quantification and ion chromatography coupled with amperometric analysis for BCAAs quantification.

In pre-digestion fermented samples, the combination of the two microorganisms showed the best fermentation performance at 12 and 18 hours, producing the highest concentrations of free amino acids. Analysis of individual strains revealed that *L. plantarum* E676 released high amounts of valine, whereas *L. helveticus* 1386 produced more leucine. Post-digestion analysis revealed a significant increase in isoleucine levels, which were likely derived from the peptides generated during fermentation and subsequently broken down in the gut. This suggests that whey fermentation not only releases free amino acids and BCAAs directly but also produces peptides that serve as substrates for further amino acid release upon digestion. Among the strains tested, *L. helveticus* 1386 was particularly effective in enhancing the bioavailability of peptides during gut digestion, indicating its potential role in improving protein utilization. Results showed that targeted whey fermentation could transform this dairy by-product into a high-value ingredient for specialized nutritional formulations.

An in-silico prediction pipeline for data mining of antifungal peptides for potential applications as food preservatives

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Globally consumed bread deteriorates fast, mostly because of mold contamination. Microbial spoilage by molds still remain responsible for huge economic losses in the bakery industries. Fungal development on bread also poses health risks due to potential mycotoxin production. Routinely, salts of propionic, sorbic and benzoic acids are used as the chemical preservatives. However, concerns regarding their safety and potential for microbial resistance have spurred research into natural alternatives, also due to general sensory dissatisfaction and low awareness in the food market. Currently bio-preservation deserves an increasing interest for food industries, including bakeries. We aim to develop novel and natural solutions for bread bio- preservation based on the combination of bioactive peptides and plant antimicrobial compounds. Multiple databases are available where bioactive peptides from different sources are classified based on antifungal, antimicrobial, antioxidant, antihypertensive, etc. A selection peptide pipeline was developed based on several parameters such as sequence length (cutoff at 5-30 amino acids), net charge, hydrophobicity (using PTPAMP), amphipathicity (using antifp), haemolytic activity (using tools like happen and hemopred), Gibbs free energy (using ΔG predictor), and MIC (using antifungipept). The dataset collected using the above parameters were visualized using heatmap and k means clustering to choose the potential antifungal peptides. Peptides were sequenced aligned using Multialin tool for identification of similar amino acid residues and subjected to key motifs discovery using MEMESUITE to highlight specific characteristics. The pipeline was also used for selection of plant matrices to extract bioactive peptides and other antimicrobial compounds using enzymatic treatment. *In vitro* antifungal assays such as hyphal radial growth test, spore germination count, growth kinetics were used to screen for activity against indicator fungus *Penicillium roqueforti* and other molds frequently isolated in bakery environment.
After screening, the selected compounds were applied in bread recipe for long term shelf-life test as novel preservatives.

Phytochemical content, antimicrobial and anti-biofilm activities of pineapple (*Ananas comosus*) peel extracts against *Pseudomonas syringae* pv. *tomato*

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Pseudomonas syringae pv. *tomato* (Pst) is the causal agent of bacterial speck disease in tomato, leading to significant yield losses. Plant-derived extracts have gained attention in the management of *Pst* because they provide a sustainable and eco-friendly alternative to synthetic chemicals. Many extracts are rich in bioactive compounds such as phenolics which can inhibit bacterial growth, reduce disease severity, and enhance plant defense responses. Unlike conventional pesticides, plant-based extracts are biodegradable, pose lower risks to human health and the environment, and may reduce the emergence of resistant bacterial strains. Therefore, they represent a promising strategy for integrated disease management in tomato cultivation. This study investigated the antimicrobial and antibiofilm activities of ethanolic (ACE) and aqueous (ACD) extracts of pineapple peel, a phytochemical-rich byproduct, against *Pseudomonas syringae* pv. *tomato* (*Pst*). Microwave-assisted extraction (MAE) was applied as an ecofriendly method, using 80% ethanol and distilled water as solvents, and the extracts were concentrated by rotary evaporation. The total phenolic content was determined using the Folin–Ciocalteu method. Antimicrobial activity was assessed through inhibition zone measurements and bacterial growth assays, while antibiofilm efficacy was evaluated against mature *Pst* biofilms formed in polystyrene 96-well plates using a biofilm inhibition assay. Furthermore, the presence of tannins, coumarins, saponins, and quinones in the extracts was confirmed by biochemical tests. Ethanolic extract exhibited stronger anti-Pst activity than the aqueous extract, particularly with respect to antimicrobial (inhibition zone diameters of ACE: 10 mm; ACD: 8 mm) and antibiofilm effects (ACE extract achieved 100% inhibition at 1/8 dilution, whereas ACD extract achieved 100% inhibition at 1/4 dilution). Total phenolic contents were determined as 1.92 and 1.47 mg GAE/g in ACE and ACD extracts respectively. Quinone and coumarin compounds were identified in both ACE and ACD extracts based on biochemical assays. These findings indicate that pineapple peel extracts exhibits notable antimicrobial and anti-biofilm activities against Pst, highlighting its potential as a natural product for the biological control of plant pathogens. Pineapple peel extracts were demonstrated to be a promising and economical source of bioactive and functional molecules for mitigating bacterial speck in tomato plants, helping to preserve quality.

Exploitation of lactic acid bacteria for the production of a fermented lupin-based beverage

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Legumes are a beneficial component of the human diet and their nutritional benefits are currently being re- evaluated along with the health-promoting potential of legume-based fermented foods and beverages.
Among legumes, lupin has recently gained scientific interest due to its high protein and dietary fiber contents, reduced fat and richness in bioactive compounds. However, the lupin consumption is limited by the presence of several anti-nutritional factors, such as alkaloids which have toxic effects in mammals. Among the approaches used to remove alkaloids, biological methods involving the inoculation of selected fermentative strains can represent a successfully alternative to traditional methods for improving the nutritional value of lupin.
The aim of this study is to screen a collection of 24 lactic acid bacteria strains belonging to *Lactiplantibacillus plantarum*, *Lacticaseibacillus paracasei*, *Leuconostoc mesenteroides* and *Lacticaseibacillus rhamnosus*, isolated from fermented foods, for producting a lupin-based fermented beverage. Primarily, the protocol for obtaining the beverage from lupin flour was optimized. Then, single- strain fermentations were performed for 24 hours, monitoring physico-chemical and microbiological parameters (pH, titratable acidity, carbohydrate/organic acid concentrations and cell viability). In order to evaluate the effect of fermentation on the alkaloid profile, the main lupin alkaloids (lupanine, sparteine, multiflorine, albine, angustifoline, hydroxylupanine) were quantified using HPLC-MS/MS. Overall, the results confirmed that all strains were able to ferment the lupin-based beverage within 24 hours while maintaining a high viability (10^9 CFU/ml), indicating that the matrix is a suitable substrate for fermentation. Moreover, all the tested strains demonstrated the ability to reduce the total alkaloid content in a strain-specific manner and, for some of them, the residual levels were below the maximum legal limit (200 mg/kg). These findings support further investigation to study additional functional activities of fermentation by these selected starter cultures and attempt to elucidate the mechanisms potentially involved in microbial alkaloid degradation.

High Hydrostatic Pressure treatment of fresh pork sausages: effects on microbiological shelf-life and safety

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Fresh sausages are unfermented meat products obtained by mixing lean and fatty pork meat with NaCl, pepper, spices. The mixture is stuffed into natural/synthetic casing, packed under modified atmosphere or vacuum and stored at refrigerated temperatures for maximum 12-15 days. Due to the intrinsic product characteristics (pH close to 6, high water activity), shelf-life and safety are guaranteed by storage at 0-4°C, often difficult to maintain. High Hydrostatic Pressure (HHP) has been proposed as an adjunctive hurdle to control microbial proliferation, since alone cannot be considered a sterilization method. In this study the effect of a 5-minute HHP treatment at 600 MPa on microbiological features was assessed. In particular, the evolution of main microbial groups (total mesophilic count TMC, lactic acid bacteria LAB, staphylococci) was monitored in relation to different storage conditions including thermal abuses before HHP treatment or during storage. In addition, challenge tests with *Salmonella* Enteritidis, *Staphylococcus aureus* and *Listeria innocua* were performed. The results showed an increase in shelf-life up 90 days in HHP treated samples (compared to 15-20 days for conventional products). Concerning thermal abuses, an overnight storage at 15°C before HHP affected TMC of untreated samples, while in HHP treated sausages was negligible. The storage at 8°C after HHP strongly influenced TMC and LAB counts, with values higher than 7 log CFU/g after 60days, while in not-thermally abused sausages (4°C) they remained below 4 log CFU/g. According to the challenge tests carried out, HHP treatment was effective against S. Enteritidis, while *L. innocua*, whose cells counts were always below the detection limit during storage, was qualitatively detected after 90 days. *Staph. aureus* was most resistant, with a 3-log reduction due to HHP, followed by stable values during storage. Further analyses are in progress to evaluate the effects of HHP on colour and texture parameters.

Developing microbial consortia to produce new plant-based fermented foods leveraging microbial biodiversity from traditional products

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In recent years, the growing awareness regarding environmental and health impacts of animal-based products has led to an increasing demand for new plant-based foods. Exploitation of strains from traditional fermented foods is a promising strategy for food industry to produce high nutritional value and functional foods.
In this project more than 100 microbial strains were isolated from traditional salami and screened by genotypic techniques, such as Amplified Fragment Length Polymorphism (AFLP), and characterized for antibiotic resistance and biogenic amine production. As a result, 15 strains belonging to *Staphylococcus* genera were selected for food application.
Metabolic profiles of these strains were then tested using BiologGen III microplates to establish their ability to utilize 71 different carbon sources. Based on metabolic complementarity, 8 bacterial strains were selected to perform fermentations, co-cultured with selected lactic acid bacteria strains, on sterilized white beans, chosen as matrix due to their high proteins content. After 24, 48 and 120 hours of incubation, pH measurement and total microbial load were carried out to assess strains capability to develop in the substrate. Finally, 5 different consortia were tested under various fermentation conditions (salt addition, time, temperature) to obtain a product with interesting organoleptic features. This study shows how microbial strains isolated from animal-based foods could be successfully employed in fermentation process and, lately, in the production of alternative plant-based foods.

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Strains of *Lactiplantibacillus plantarum* belonging to the microbial collection of Porto Conte Ricerche were selected for the production of exopolysaccharides in durum wheat middlings

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Microbial culture collections are a valuable resource in the field of research. One of the most common practices involves creating internal laboratory strain banks, aimed at establishing a true archive of microbial cells. Currently, the microbial strains belonging to the Porto Conte Ricerche collection have been isolated and identified as follows: 67% from sourdoughs used in the production of traditional breads, all originating from different areas of Sardinia, and the remaining 33% from the dairy sector.

Approximately 1,000 strains, including lactic acid bacteria and yeasts, have been isolated and identified. Some of them have been studied with the aim of selecting starter strains useful in fermentation processes. The ability of a total of 207 *L. plantarum* strains to produce exopolysaccharides (EPS) was evaluated in a plate assay. A modified MRS medium containing 5% of four different carbon sources (maltose, fructose, sucrose and glucose) was used. The EPS phenotype was confirmed by the formation of long strings or the presence of mucilaginous material on the colony. This initial test enabled the most prolific EPS-producing L. plantarum strains to be inoculated into semolina and durum wheat middlings (*tritello*) based doughs. After 16 hours of incubation at 25 °C the doughs viscosity was examined, and pH and TTA (total titratable acidity) were measured.

The analysis revealed that the selected strains produced a greater amount of organic acids in *tritello* doughs than in semolina doughs, being the same pH value. Analysis of viscosity revealed a strain (PCC 2397) isolated from sourdough differing in terms of cohesiveness and viscosity. This microorganism might be used as a starter to prepare sourdough with grain milling by products, in order to improve the dough and the bread structure.

Enhancing the antimicrobial potential of hops using natural deep eutectic solvents

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Foodborne diseases are a significant cause of morbidity and mortality worldwide, as well as being a major impediment to socioeconomic development. Furthermore, non-pathogenic microorganisms can pose a significant challenge as they can diminish the quality of contaminated food products. To mitigate the risk of foodborne diseases and food deterioration, various food preservation methods have been employed over the years. Among these, new natural antimicrobials derived from plant sources are attracting the attention of consumers and industry.

In this study, extracts from vitro-derived hop (*Humulus lupulus* L., cv. *Columbus*) plantlets were evaluated for their antimicrobial activity, which depends on the phytochemical profile and concentration of active compounds. Innovative extraction strategies using natural deep eutectic solvents (NaDES) were explored to obtain effective, eco-friendly and high-performance extracts. A Design of Experiment (DoE) approach was employed to evaluate the influence of various extraction parameters on the total phenolic content (TPC) and the antimicrobial activity of the extracts. Two qualitative factors, the type of hydrogen bond donor and acceptor components in the NaDES, and one quantitative factor, the hydrogen bond acceptor to donor ratio, were evaluated. Data analysis using Modde software identified the key factors that influence extract performance in terms of TPC and antimicrobial activity. The results were also compared with those obtained using a conventional ethanol/water extraction method, highlighting the potential of NaDES as a promising method for obtaining antimicrobials from plant matrices.

Discovering the core microbiome of table olives

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Vegetable fermentations are part of the tradition of many countries. Archaeological records show that table olives have been produced in the Mediterranean basin since 6500 years ago. Production of table olives is concentrated in the Mediterranean Basin, but it is expanding in South America. As in other vegetable fermentations, the lack of heat treatments, the reliance on natural contamination and on selective factors (NaCl, anaerobiosis, occurrence of food phenolics, etc.) determine the dynamics of the microbial communities and, ultimately, the sensory properties of the product. Lactic acid bacteria (LAB) and yeasts have a pivotal role in table olive microbial communities, but halophilic and alkalophilic microorganisms may also contribute, positively or negatively, to the quality. We have combined metataxonomic data from the FoodMicrobionet database with those of a large survey carried out in our laboratories to provide quantitative insights on the structure of bacterial and fungal microbial communities of table olives and to identify core genera in different trade preparations and varieties.

A set of 30 bacterial and 30 fungal genera may be representative of the dominating core microbiota. *Celerinatantimonas* and *Lactiplantibacillus* were the most prevalent bacteria, followed by several LAB, halophilic and alkalophilic lactic acid bacteria (HALAB) and Gram negatives, including non-halophilic species. Similarly, 3 fungal genera (*Pichia, Candida* and *Wickerhamomyces*) were the most abundant and prevalent.

Distribution of both bacteria and yeasts varied significantly in different olive varieties, among olives, brines and contact surfaces or materials, and different production stages. No clear grouping related to the combination of ripeness and trade preparation was found, although HALAB were characteristically abundant in Spanish style green olives and Picholine. Addition of starters affected the composition and dynamics of microbial communities to a variable extent.

This study may contribute to the design of novel, mode competitive starters based on microbiome based approaches.

Influence of wooden table biofilms on microbial dynamics in artisanal cheese-making

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Wooden equipment in artisanal cheese-making is increasingly recognized for supporting complex microbial biofilms that impact the organoleptic qualities and safety of the final products. In Sicilian stretched-curd cheeses, wooden tables are used during acidification and moulding. This study investigated the microbial transfer from the wooden table (WT) biofilm to curd during acidification, compared to stainless steel surfaces (ST). Before acidification, curd was split into two equal portions and placed on either WT or ST overnight. Culture-based analysis of fermentation-associated, spoilage, and pathogenic microorganisms was performed to establish their presence and quantity in curd before acidification, and after acidification on a wooden (CW) or steel table. The impact of surface type on microbial transfer and microbiota composition was evaluated using linear mixed-effects models. To investigate taxonomic differences and potential microbial transfer from the biofilm of WT to CW, the metagenomic DNA from all samples was sequenced targeting the V3 region of the 16S rRNA gene and ITS regions of *Lactobacillus* and fungi. Acidification on WT resulted in significantly greater reductions in undesirable microbial populations than on ST, particularly for Enterobacteriaceae, coliforms, *Escherichia coli*, and *Staphylococcus aureus* (>1 log CFU/g, p <0.4). Yeast populations did not differ significantly between surfaces, nor did other microbial groups show substantial variation. WT was found to transfer to the curd *Corynebacterium* and a diverse array of *Lactobacillus* species. While *Lactobacillus delbrueckii* dominated acidification in both conditions, CW exhibited a richer lactic acid bacterial community. Core taxa included *Lactobacillus kefiranofaciens* and *Lactobacillus paracasei*, with transient taxa such as *Limosilactobacillus fermentum*, *Lactobacillus gasseri*, and *Lactobacillus helveticus*. The fungal community consistently included *Saccharomyces cerevisiae* transferred from the WT to curd. These findings support the role of wooden equipment as a microbial reservoir that shapes the fermentative ecosystem of stretched-curd cheeses, highlighting its importance for both cheese quality and safety.

Exploring the genomic biodiversity of *Lactococcus lactis* from mountain cheeses for application in microencapsulation and cheesemaking

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Lactococcus lactis is a key bacterial species in dairy fermentations, contributing to acidification, flavor development, and microbial stability in cheese. While industrial strains are well characterized, *L. lactis* populations from artisanal and natural environments remain underexplored, despite their potential to enhance the genetic and functional diversity of starter cultures. This study investigated the genomic diversity of 27 *L. lactis subsp. lactis* strains isolated from traditional mountain cheeses produced in Trentino (Italy), via spontaneous fermentation in microbiologically closed environments where local microbial ecosystems naturally evolve. These niche conditions provide a unique opportunity to explore native biodiversity and its potential food applications. Our comparative pan-genomic analysis identified a total of 5,765 genes, including 1,767 core, 2,818 accessory, and 1,180 unique genes. Key findings include genes involved in stress response (e.g., thioredoxins, cold shock proteins), flavor compound production (e.g., acetoin and diacetyl biosynthesis pathways), and antibacterial activity (e.g., nisin biosynthesis via the NisK-NisR regulatory system). Carbohydrate metabolism was well represented, with conserved transport and catabolic systems for glucose, fructose, lactose, and mannose, essential for growth and acidification in milk. Importantly, the accessory genome contained genes related to phage resistance, environmental adaptation, and metabolic flexibility, highlighting the evolutionary dynamics of *L. lactis* in artisanal cheese environments. The rich functional diversity uncovered in these wild strains suggests promising opportunities for their incorporation into cheese production. Moreover, their metabolic versatility and resilience make them potential candidates for encapsulation in microcapsules aimed at targeted delivery of bioactive compounds in complex food matrices. Our findings underscore the value of biodiversity-driven approaches for developing robust, multifunctional starter cultures and bioactive carriers to support innovation in the production of high-quality, naturally fermented foods.

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Quality and shelf-life enhancement of orange- bergamot beverage through high-pressure treatments

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Citrus-based beverages are highly valued for their refreshing taste, rich nutritional profile, and abundant bioactive compounds such as antioxidants and phenolics. However, their quality and shelf-life are often compromised by microbial spoilage and physicochemical changes during storage. Traditional thermal pasteurization methods can ensure safety but often degrade sensory and nutritional properties. Recently, non- thermal high-pressure technologies, including High-Pressure Homogenization (HPH) and High Hydrostatic Pressure (HHP), have gained attention as innovative approaches to maintain product freshness while providing microbiological safety.

This study investigates the effects of HPH and HHP on the quality and shelf-life of an innovative orange- bergamot beverage during refrigerated storage. The beverage samples were subjected to HPH at 200 MPa for three cycles and HHP at 600 MPa for 100 seconds, with pasteurized and untreated juices as controls. Shelf-life was assessed over 19 days at standard refrigeration (4°C) and mild thermal abuse conditions (8°C) by monitoring microbiological stability, antioxidant activity, total phenolic content (TPC), volatile compound profiles, pH, and color stability. Results demonstrated that both high-pressure treatments, along with thermal pasteurization at 80°C for 15 minutes, effectively maintained microbial stability for 19 days at both temperatures, whereas untreated samples spoiled within 13 days at 8°C. Challenge tests with spoiling microorganisms *Hanseniapora occidentalis* and *Lactiplantibacillus plantarum* showed that HPH and HHP reduced microbial loads by over five logarithmic cycles. Additionally, HPH and HHP preserved the antioxidant capacity, volatile aroma compounds, color, and phenolic content significantly better than thermal pasteurization and untreated controls, which exhibited marked deterioration over storage. In conclusion, HPH and HHP are promising non-thermal technologies that enhance the shelf-life and quality of citrus-based beverages by effectively controlling microbial spoilage while preserving key bioactive and sensory attributes, offering a viable alternative to conventional pasteurization.

Determination the gastrointestinal survivability of probiotic *Lactobacillus pentosus* microcapsules

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Microencapsulation is an effective technique for stabilizing and maintaining the viability of probiotic microorganisms under stress conditions. In this study, *Lactobacillus pentosus* cells were encapsulated using different coating materials-reconstituted skim milk (RSM), gum arabic (GA), and maltodextrin (MD)-via the freeze-drying method. The resulting microcapsules were evaluated for their ability to enhance probiotic survival under various stress conditions, including simulated gastrointestinal environments. The survivability of encapsulated cells were assessed in simulated gastric and intestinal fluids. Among the tested formulations, microcapsules prepared with the RSM:GA combination demonstrated significantly higher viability. A reduction of approximately 2-3 log CFU/mL in viable cell counts was observed under both gastric and intestinal conditions. These findings suggest that the RSM:GA coating matrix offers superior protection to
L. pentosus during gastrointestinal transit. The use of naturally sourced, food-compatible coating agents such as RSM:GA enables the development of clean label products. Microencapsulation allows for targeted release of probiotics (capsules that open in the intestine or stomach), which is an important step in personalised nutrition approaches.

Valorization of almond skins as prebiotic additives in synbiotic beverages and microcapsules

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Almond by-products represent a valuable source of nutrients and other functional substances with potential for use in food and nutraceutical formulations. Almond polyphenols, for example, may promote gut health by modulating the microbiota, an effect further supported by the high content of dietary fiber and other bioactive compounds. This research has been dedicated to reshaping almond skins into food ingredients with valuable functional properties. The main goal was to develop functional drinks and microcapsules enriched with dehydrated almond skins as prebiotics and *Lactobacillus acidophilus* as a probiotic.

First, the prebiotic effect of different concentrations of dehydrated skins (2.5%, 5%, and 7.5%) was evaluated on strain viability and acidification during storage at 4, 15, and 30°C for 100 days. Subsequently, two *in vivo* applications were performed. A soy-based drink supplemented with *L. acidophilus* (8-9 log cfu/g) and 2.5% of almond skins was produced and refrigerated at 4 °C for 21 days to evaluate shelf-life.

Concurrently, a microencapsulation process was optimized to co-encapsulate the selected probiotic, almond flour, and skins to enhance stability and functionality. The encapsulated probiotic was evaluated for its viability under simulated gastrointestinal conditions and for tolerance to some environmental stressors (freezing, thermal treatments, salinity, and exposure to organic acids).

Results indicated that almond skins supported probiotic persistence and acidification kinetics, suggesting their suitability as a prebiotic additive in synbiotic formulations. In controls, the microbial population showed a significant decline (< 6 log CFU/mL after 50 days), whereas skin-enriched samples exhibited a slower death rate (~7 log CFU/mL after 100 days). The prebiotic effect was also confirmed in non-dairy drinks. Moreover, the formulation containing 2.5% sodium alginate, 3% almond flour, and 3% almond skin was identified as optimal for *L. acidophilus* entrapment, ensuring high encapsulation efficiency, effective protection during storage and stress exposure, and controlled intestinal release.

Harnessing biodiversity in dairy cultures: fifty years of *Lactobacillus helveticus* evolution

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This study investigates the genetic and phenotypic biodiversity of *Lactobacillus helveticus* (LH) strains isolated 50 years apart from natural whey starters (NWS) in traditional dairy productions and never revitalized, in comparison with recently isolated LH strains from the same ecological niche. A preliminary *in vitro* screening of 40 LH strains (previously tested for the absence of antibiotic resistance and amino- biogenic properties) revealed significant biodiversity between the two groups. Older strains showed higher growth rates and shorter lag phases when cultivated in both broth medium and milk under traditional fermentation conditions, suggesting better fitness and adaptation. To evaluate LH technological potential, the most promising strains were tested in a miniaturised cheese model, simulating the traditional process conditions. Older strains showed decreased cell viability and produced distinct volatile compounds after two weeks of ripening, with higher levels of alcohols and lower amounts of acetic acid and acetoin. Based on these results, two old and two recent strains were selected for pilot-scale cheese trials, co- inoculated with *Lactobacillus delbrueckii* subsp. *lactis* UPCCO5365 to mimic traditional NWS cultures using a defined protocol for cooked hard cheese production. The samples (approx 1 kg) were ripened for three weeks at 18°C. Culture-dependent and culture-independent techniques were applied to study microbial dynamics showing strain-specific differences. In addition, SPME-GC-MS demonstrated distinct aroma profiles, highlighting different metabolic performances within dairy fermentations. This work offered a solid base for the valorization of LH strains in tailored dairy applications, providing important insights into the adaptive evolution of this species in these environments.

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Sustainable utilization of Carasau bread residues through microbial fermentation

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Carasau bread is a traditional thin and crispy flatbread produced in Sardinia from durum wheat semolina, water, and baker's yeast or sourdough. Its production involves a double-baking process at high temperatures following the dough leavening. During the first baking, the dough sheet puffs up due to CO_2 expansion and water evaporation, forming a double-layered bread known as *pane lentu*. The layers are separated by trimming the edges, and the resulting sheets undergo a second baking, to become Carasau bread. The trimmed edges, or residues, constitute production waste and are typically used as animal feed. However, microbial fermentation presents an opportunity to reintegrate these residues into the food production chain. In this study, Carasau bread residues were dried, grinded, and used as a substrate for sourdough fermentation. Three types of substrates were prepared: (i) bread residues alone, (ii) bread residues with the addition of wheat middlings (*farinaccio*), and (iii) semolina. The substrates were fermented using selected strains of *Lactiplantibacillus plantarum*, *Saccharomyces cerevisiae* and *Wickerhamomyces anomalus*, which belong to the Porto Conte Ricerche microbial collection. Fermentations were conducted at 25 °C for 8 h in a bioreactor (AFTL5, SITEP S.r.l., Voghiera, Italy). Chemical, physical and microbiological analyses revealed that the substrate composed of bread residues and *farinaccio* produced comparable results to the semolina- based substrate, with regard of acidity and microbial population dynamics over five days of backslopping.

These findings demonstrate that microbial fermentation offers a viable and sustainable strategy for valorizing bread residues. The resulting fermented substrate is suitable for use as sourdough in the production of Carasau bread.

All bio-based μ-beads from microalgae for future probiotics delivery

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The development of sustainable delivery systems for probiotics is a key challenge in the food and health fields, driven by the aim to enhance quality, nutritional and functional properties while ensuring eco-friendly transformation processes. A key issue with certain health-promoting bacteria is their low resistance to industrial processing, storage, and gastrointestinal (GI) transit, which can significantly reduce their viable cell counts.
In this study, we introduce a fully bio-based system for encapsulating and protecting probiotics, built upon the naturally structured silica micro-shells of the diatom *Coscinodiscus granii*. The porous biosilica micro- containers were properly processed to obtain suitable 3D-biosilica containers, which were then loaded with a commercial mixture of probiotic bacteria (*Lacticaseibacillus rhamnosus* IMC 501 and *L. paracasei* IMC 502) through a mild vacuum-assisted process and subsequently sealed with two natural enteric biopolymers, Shellac and Chitosan, taking advantage of their electrostatic interection. The resulting hybrid microcapsules achieved an encapsulation efficiency of 71 ± 7%. Notably, the combination of biosilica and biopolymers significantly enhanced bacterial survival under simulated GI conditions, thermal stress (up to 55°C), and prolonged dehydrated storage at 4°C. Compared to control samples, the system maintained higher microbial viability, supporting its suitability for targeted delivery to the colon. This approach exploits the diatoms' intrinsic morphology and porosity, eliminating the need for complex encapsulation technologies. The interplay between biosilica and polymer coatings creates a scalable, eco- compatible platform that isolates microbes from environmental stressors. Altogether, our findings highlight *C. granii*-based microcapsules as a promising green solution for the controlled delivery of live probiotics in functional foods and dietary supplements.

Development of functional symbiotic beverages enriched with pomegranate peel for enhanced health and sustainability

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Nowadays, companies are increasingly seeking functional foods providing health benefits to consumers, while supporting environmental sustainability. In this context, novel beverages were formulated by mixing apple juice/pulp with different plant-based drinks, i.e. oat, spelt, or soy, or whole milk. Formulations were supplemented with probiotic bacteria (*Lactiplantibacillus plantarum* DSM 25710 and *Bifidobacterium lactis* BB12, inoculated alone or in combination), along with pomegranate peel (PP), a by-product rich in tannins with antioxidant, antimicrobial, and gut health-promoting properties. To assess the effects of both the PP and microbial strains - alone and in synergy - cell viability, total phenolic content, antioxidant capacity, and volatile compound profiles were monitored over 40 days at 4°C. Results indicated that *L. plantarum* maintained high viability throughout the storage period, regardless the presence of PP or the type of beverage. Conversely, *B. lactis* showed a marked reduction in viability after 20 days, enhanced by the presence of PP. Notably, co-culturing the strains improved *B. lactis* survival until the end of the storage, being the viability of both the probiotics improved in the presence of PP. Additionally, PP inclusion resulted in a threefold increase in phenolic content and a tenfold boost in antioxidant activity. Finally, beverages containing *L. plantarum* displayed a diversified and distinct volatile profile, suggesting a greater metabolic activity.

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Reduction of alcohol content in Nebbiolo wines using *Schizosaccharomyces japonicus* yeast

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Global climate change, along with the consequent production of increasingly alcoholic wines, poses several technical challenges for winemakers. Simultaneously, the growing public awareness of the health risks caused by excessive alcohol consumption has led to an increasing interest in strategies aimed at producing wines with lower alcohol content. While the subtractive approach to reducing wine ethanol through physical processes is well-documented, the potential of employing fermentative yeasts that naturally produce lower alcohol levels has not yet been fully explored.

In this study, we present preliminary results obtained using a non-*Saccharomyces* strain in sequential inoculation with *Saccharomyces cerevisiae* ISE 77 strain on Nebbiolo grapes from Piedmont. An initial screening of several non-*Saccharomyces* yeasts from the culture collection CREA-CMVE, highlighted the favourable oenological characteristics of ISE 1452, *Schizosaccharomyces japonicus* strain, such as efficient sugar consumption, low volatile acidity production, and minimal nutritional requirements. In the first part of this work, the co-inoculation method was optimized; subsequently micro-vinifications were conducted and compared with single ISE 77 *S. cerevisiae* strain inoculation.

The wines obtained through sequential inoculation showed a reduction of almost one degree in ethanol content, 12.43±0.02 v/v%, compared to those fermented with only *S. cerevisiae* strain 13.30±0.03 v/v%. They also contained nearly twice the glycerol concentration and lower volatile acidity. Moreover, the analysis revealed a marked malo-alcoholic metabolism of the ISE1452 strain, which was able to partially consume malic acid during fermentation.

Wines produced via sequential inoculation exhibited significantly higher total anthocyanin indices and greater color intensity. In contrast, flavonoid levels tended to be higher in monosporial fermentation with the ISE 77 *S. cerevisiae* strain. The analysis of the volatile components revealed differences between the wines; the sequential inoculation enhanced the production of higher alcohols and acetate esters, such as isoamyl acetate, which contribute to the fruity notes and overall aromatic complexity.

Effect of freeze-drying on composition and metabolic functionalities of natural cheese whey starters

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Natural cheese whey starters (NWS) are used for the production of several typical and branded products. NWS are often produced artisanal, in small dairies, and the variability of the operative conditions may compromise the structure and functionality of microbial consortia; therefore, the preservation of NWS microbiomes is desirable to ensure the reproducibility and quality of dairy production. We investigated the effect of freeze-drying (FD) and long-term storage (up to 9 months) on the survival (plate counting), composition (AT-HTS sequencing, using both 16S rRNA and ITS2 as targets), and functionalities (acidification rate, SPME-GC/MS) of microbiomes of 2 from Basilicata region. A revitalization protocol, based on backslopping procedure, was also applied at the end of long-term storage (9 months) to restore viability and functionalities of NWS (plate counting, AT-HTS, acidification, SPME- CG/MS). A wide microbial diversity was found among the unprocessed NWS. The FD process had the greatest effect on microbial survival, and further reduction of viability was observed after 3 months of preservation. Long- term storage (6 and 9 months) did not further affect NWS survival (reduction did not exceed 2 log cycles). Metataxonomic analyses revealed that FD process and long-term storage did not significantly affect the macrostructure of NWS microbiota. On the contrary, the acidification rate was impaired. The reconstituted samples, however, regained a good acidifying capability after 3 backslopping cycles, and the volatilome profile became more complex, suggesting that proper propagation procedures are crucial to restore the functionality of microbial communities. The results confirm the effectiveness of FD in long-term storage of NWS. Further studies, however, are needed to verify the reuse and the performances of preserved NWS in food matrices.

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NIR and chemometrics for sourdough bread authentication: enabling traceability in resilient food supply chains

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Research on fraud detection in the bread and bakery market, especially traditional sourdough, remains limited. The lack of regulations and official authentication methods creates a significant gap, given sourdough's high market value due to production methods and premium ingredients. This prompted the development of rapid, reliable methods combining modern analytical instrumentation, chemometrics, and machine learning to ensure authenticity and protect the agri-food chain. Eighty experimental bread prototypes were produced and characterized in triplicate at laboratory scale, analyzing technological variables and production dynamics of artisanal and industrial processes. Samples comprised various proportions of Type I, II, and powdered commercial Type III sourdough, alongside control breads made with fresh baker's yeast, the biga method, and chemically acidified doughs simulating fraud practices. NIR spectra of crust and crumb of the experimental samples were collected in transflectance mode using a Nicolet iS50 spectrometer equipped with an integrating sphere (4000–12500 cm^{-1}) and OMNIC software (Thermo Fisher Scientific). Multivariate analysis using PCA and PLS-DA was performed with Solo v9.3.1 for classification. PLS-DA clearly distinguished sourdough from non-sourdough breads. Spectral data showed similar distribution patterns for crust and crumb. VIP scores identified spectral regions between 5000–5500 cm^{-1} and 7000–7500 cm^{-1} as crucial for discrimination. The study is currently being implemented, with model validation planned to involve commercial bread samples directly obtained from industrial and artisanal producers. Further investigations will focus on identifying the compounds responsible for absorption in key discriminative spectral regions.

Cinnamon meets probiotics: enhancing functional chocolate with *Lacticaseibacillus rhamnosus* CRL1505

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Functional foods go beyond providing essential nutrients as they are enriched with bioactive compounds like antioxidants (e.g., phenolic compounds, carotenoids, and vitamins) and probiotics that support health and help prevent disease.

This study aimed to formulate two probiotic-enriched milk chocolate pralines, with and without cinnamon, and to evaluate the impact of cinnamon on probiotic survival during six months of ambient storage and simulated digestion. The selected probiotic strain, *Lacticaseibacillus rhamnosus* CRL1505, is known for its immunomodulatory effects, particularly in preventing respiratory infections in children. Two chocolate variants were produced at an industrial scale: one containing only *L. rhamnosus* CRL1505 and the other with both the probiotic and 3.4% cinnamon powder. Both products were stored at 25°C for 180 days, with monthly analyses assessing probiotic viability, microbiological stability (molds, yeasts, *Enterobacteriaceae*), and physicochemical properties (pH, water activity). Probiotic survival during simulated gastrointestinal transit was evaluated at 0, 3, and 6 months using the standardized INFOGEST *in vitro* human digestion static protocol.

Both chocolate formulations remained microbiologically and physicochemically stable throughout the six- month period, with no detectable molds, yeasts, or *Enterobacteriaceae*. *L. rhamnosus* CRL1505 maintained high viability under ambient storage conditions. In the cinnamon-supplemented chocolate, probiotic counts were comparable to the control for up to four months. By the end of storage, viable counts exceeded 7 log CFU/g in the control and 6 log CFU/g in the cinnamon variant. Simulated gastrointestinal digestion showed that the probiotic retained viability through all digestive phases, with survival rates above 75% and an average reduction of 1 log CFU/g after six months of storage, at the end of the shelf-life.

In conclusion, *L. rhamnosus* CRL1505 showed high stability in milk chocolate with cinnamon and, due to its respiratory health benefits, is ideal for child-focused functional products using natural ingredients.

Effect of a thermal abuse on the viability of functional microorganisms in a "fiordilatte" ice-cream

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The use of non-conventional matrices as carriers for probiotic microorganisms is an increasing trend in food microbiology and technology, strictly connected to the new function of foods and to their role as driver or markers of lifestyle and well-being. In this context, ice cream represents a valuable carrier for functional microorganisms; however, while there are many studies focusing on the optimization of inoculation, and viability of probiotics during storage, there are a few evidence on the effect of a thermal abuse during preparations and early lifetime, considering that an increase of temperature and a partial defrosting of product usually occurs during its lifetime as a consequence of improper logistic and/or handling.
Thus, two functional microorganisms (*Lactiplantibacillus plantarum* c19 and *Bifidobacterium longum* subsp. *infantis* DSM 20088) were inoculated in a "fiordilatte" ice cream, by modulating the duration and the numbers of thermal abuses/defrosting (from 2 to 6 h, and 1-3 times, respectively), and the age of cells (15, 24, and 72 h) through a full factorial design. Then, the samples were stored at -18°C and periodically analyzed over 4 weeks. Data were analyzed through multifactorial ANOVA and multiple correlations.
Cells age was significant for both microorganisms, with a negative effect on the viability after a thermal abuse when cell age increases, although the weight was different depending on the microorganism; also, the number or the duration of defrosting could play a role, less in *Lpb. plantarum* and more in *B. infantis*.
During 4-week storage, the microorganisms did not experience further decreases in the viable count, thus confirming that the first steps or a thermal abuse at the beginning of the lifetime could significantly affect the residual concentration of a probiotic in the product.

Metabolomic profile of pomegranate cider-like beverages started by different yeast species

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This study aimed to assess the differences, in terms of physico-chemical characteristics, antioxidant properties and flavour profile, among four pomegranate cider-like beverages (PCB), produced starting from two pomegranate juices, fermented by either commercial *Saccharomyces cerevisiae* or a non-conventional, autochthonous strain of *Hanseniaspora valbyensis*.

Although both starters were able to drive the fermentation of pomegranate juices, *H. valbyensis* reached higher cell densities four days earlier than *S. cerevisiae*. After fermentation, PCB were subjected or not to a clarification treatment (centrifugation or filtration) and matured for additional seven days. In the mature PCB, filtration caused a stronger decrease in yeast cell density compared to centrifugation. As for elemental composition, K and Ca increased during fermentation, whereas Fe and Cu decreased. A strong reduction of Cu, Zn, as well as Fe and Ni, was observed in clarified mature PCB. The use of *H. valbyensis* led to a more rapid consumption of monosaccharides, higher acetic acid and lower ethanol concentrations compared to *S. cerevisiae*.

The final profile of Volatile Organic Compounds (VOCs) was reached after three and seven days of fermentation with autochthonous and commercial yeast, respectively. After maturation, PCB obtained with *H. valbyensis* kept more complex VOCs profile, compared to *S. cerevisiae*. The panel test on centrifuged PCB did not result in any significant difference. The antioxidant activity of PCB was always high and not affected by fermentation, type of starter, and maturation. This study paves the way for better understanding fermentation capability and impact of *H. valbyensis* on food quality.

Formulation of a symbiotic blend of germinated brown rice and probiotics

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Germinated brown rice (GBR) is recognized as a sustainable cereal-derived prebiotic which can support the growth of lactic acid bacteria (LABs). GBR is rich in bioactive compounds such as γ-aminobutyric acid (GABA) and γ-oryzanol, able to exhibit estrogen-like effects in experimental model. These properties may be of interest in the context of menopause, characterized by hormonal changes and associated symptoms. Combining the use of cereal-based prebiotics with probiotics could be a promising strategy to alleviate menopause-related symptoms and support women's well-being. The aim of the present work was to investigate the synergistic effect of GBR and a probiotic blend consisting of strains ascribed to the species *Lacticaseibacillus rhamnosus*, *Limosilactobacillus fermentum*, *Lactiplantibacillus plantarum*, and *Bifidobacterium animalis subsp. lactis*. GBR was tested on vaginal VK2/E6E7 cells for cytotoxicity, by MTT test, radical scavenging activity, through DPPH assay, proliferation ability, clonogenic assay and by evaluating the Ki67 expression, glycogen release, and anti-inflammatory potential. In addition, the ability of GBR, at different concentrations (1g/ml, 0.5g/ml, and 0.1g/ml), to support the growth of LAB strains was evaluated. LABs were also tested for survival abilities in simulated gastrointestinal tract and for antagonistic activity against pathogens. Results showed that GBR did not show toxic effect and was able to exert antioxidant capacity and to significantly increase both VK2/E6E7 cell proliferation and glycogen release. Analysis of relative mRNA levels of interleukin 10 (IL-10), sirtuin-1 (SIRT-1), and IL-6 showed the modulatory effect of GBR, reflecting a significant anti-inflammatory activity. The tested LAB strains showed ability to survive under the stressful conditions occurring during the GI tract passage as well as to exert antagonistic activity against both vaginal and intestinal pathogens. Moreover, an increase in LAB cell density was observed in presence of GBR. In conclusion, GBR can be considered a valuable cereal-derived prebiotic with estrogen-like potential.

Effects of pistachio skins as dietary supplementation on fecal microbiota of healthy lambs

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The reuse of agro-industrial by-products as feed supplements in animal nutrition represents a successfully adopted strategy to reduce feeding costs, satisfy the nutritional needs of livestock, and improve sustainability, maintaining a circular economy. In this context, pistachio skins, although initially considered waste, received considerable attention as livestock feed supplement due to distinctive nutritional qualities. The aim of the present work was to investigate the impact of the dietary pistachio skins supplementation on fecal microbiota of healthy lambs using 16S rRNA gene amplicon sequencing. Lambs (n=24) were randomized into two groups, the control group (C, n=12) received the traditional diet, and the treated group (T, n=12) received 12% of pistachio skins as supplement. Fecal samples, collected at the beginning (d0) and after 58 days (d58) feeding, were subjected to DNA isolation and Illumina MiSeq analysis of the V3-V4 region of the 16S rRNA gene.
Metagenomics data revealed significant differences in alpha and beta diversity between the two dietary regimes, as well as slight variations in taxa abundance. Specifically, at phylum level, a lower abundance of *Firmicutes* and a higher abundance of *Bacteroidetes* was observed in C group then in T group. Concerning the family level, *Lachnospiraceae*, *Ruminococcaceae*, and *Verrucomicrobiaceae* mainly occurred in T group. *Akkermansia*, *Treponema*, and *Blautia* genera, short-chain fatty acid-producing microorganisms, are considered as intestinal markers of animal health, were detected only in the T group.
In conclusion, pistachio skins can be considered a valuable feed supplement for lamb's nutrition.

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Environment-driven phenotype switching of *Leuconostoc mesenteroides* GSL1 from optimum to pulse-based food-like media

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Plant-based substrates provide a natural and safe environment that closely resembles the intended industrial application of lactic acid bacteria, thereby enhancing their metabolic efficiency in food fermentation. Our study aimed to investigate the metabolic behaviour of *Leuconostoc mesenteroides* GSL1 after three sequential propagation cycles (16 h each) in faba bean- and yellow pea-based model systems, with De Man Rogosa and Sharpe (MRS) medium serving as the control. Preliminary phenotype microarray (OmniLog) analysis showed an expanded carbon source utilization in pulse-propagated strains. Gene expression analysis further confirmed this metabolic shift, with upregulated genes associated with carbohydrate transport and metabolism. Subsequently, propagated strains were employed in fermentations of the three media using a full factorial design. Notably, propagation in faba bean followed by fermentation in the same medium resulted in enhanced fructose consumption, elevated mannitol production, and reduced lactic acid yield. A differential metabolism of phenolic compounds, including catechin and epicatechin, was also found under these experimental conditions. Our findings demonstrate a growth media-dependent phenotypic plasticity in *Leuc. mesenteroides* GSL1, influencing its metabolic profile. Future integration of phenomc, metabolomics, genome analysis, and genome-scale metabolic modelling (GSMM) will enable predictive simulation of metabolic adaptation and support tailored design of pulse-based fermentations.

Study and selection of lactic acid bacteria for nut-based fermented products

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The demand for plant-based foods has favoured the development of cheese alternatives obtained through nut fermentation with Lactic Acid Bacteria (LAB), where the challenge of ensuring safety and quality relies on selected microbial consortia. The project COCONUT aims to exploit the microbial diversity of traditional fermentations for tailored processes on plant-based matrices. About 100 food-deriving LAB strains from the collections of the Universities of Parma and Bologna were studied for their genetic diversity (through Amplified Fragment Length Polymorphism), safety features (antibiotic resistance and amino-biogenic potental) and antimicrobial activity against foodborne pathogens. In addition, metabolic profiling using Biolog GENIII MicroPlates was performed, highlighting distinct substrate utilization patterns. After this screening, some strains were chosen as potential starter cultures for cashew nut fermentation, evaluating their growth and acidifying performances. Six strains belonging to different species (*Leuconostoc mesenteroides*, *Lactiplantibacillus plantarum*, *Lacticaseibacillus rhamnosus*, *Lacticaseibacillus paracasei*) were tested alone or in combinations. Nuts were heat-treated in water (75°C for 15 min), soaked for two hour and grinded to obtain a spreadable cream, that was inoculated with LAB (concentration approx. 7 log CFU/g) and fermented at 30°C overnight. The effect of increasing glucose concentrations to boost fermentation was also considered. The results showed that the strains were able to grow up to 9 log CFU/g but, in some cases, the pH did not reach 4.4, considered a safe threshold for this kind of RTE product. Better performances can be obtained by combining different strains in tailored microbial consortia, with an enrichment in the aroma profile. This research generated important knowledge in the innovative plant-based foods development, exploiting microbial diversity from traditional fermented products.

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Biocontrol agents against *Botrytis cinerea* in sustainable winemaking: impact on fermentation and wine quality

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The transition toward sustainable viticulture requires effective and environmentally friendly alternatives to synthetic fungicides. In this context, *Botrytis cinerea*, the causal agent of grey mould, represents a major challenge for grape production and wine quality, especially under increasingly restrictive regulations. This study explores the use of microbial biocontrol agents (BCAs) previously isolated from grapevine-associated microbiota—*Metschnikowia reukaufii*, *M. hawaiiensis*, and *Aureobasidium pullulans*—for the control of *B. cinerea* and their impact on winemaking. Microvinification trials were conducted on *Vitis vinifera* cv. Nebbiolo using both fresh and withered grapes. Treatments included co-inoculation of the selected BCAs with a *Saccharomyces cerevisiae* commercial strain and untreated controls with five replicates per treatment, using post-harvest grapes. These trials included fermentations performed immediately and three days after BCA application. Fermentations were carried out in 200 mL flasks with Müller valves, under standard red winemaking protocols including maceration, temperature control (20°C), aeration, and punch-downs during active alcoholic fermentation. Initial sugar concentrations significantly differed between matrices: 194.5 g/L in fresh grapes and 327.66 g/L in withered grapes. Fermentation kinetics were monitored via CO_2 release, and microbial viability was assessed at 24, 48, and 72 hours. The final sampling occurred after weight stabilization over 48 hours. Fermentation lasted 20 days in fresh grapes and 54 days in withered grapes. Wines were analyzed for key oenological parameters: pH, titratable and volatile acidity, sugar and ethanol content, organic acid profile, phenolic compounds, volatile profiles, and sensory attributes. Preliminary findings indicate that BCAs persist during early fermentation stages without significantly altering ethanol yield or core wine chemistry, even in high-sugar matrices. This research demonstrates the compatibility of selected BCAs with standard winemaking and supports their integration into sustainable viticulture practices. These findings contribute to the development of safer, regulation-compliant alternatives to synthetic fungicides for managing *B. cinerea* while preserving wine quality.

Effects of encapsulated polyphenols extracted from olive mill wastewater and olive tree leaves in modulating gut microbiota composition of broiler chickens'

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One of the main incidences in the poultry industry are gastrointestinal infections, which cause considerable economic losses. To address this problem, the use of functionalised feeds fortified with bioactive molecules with antimicrobial activity against pathogens has recently been proposed alongside pharmacological treatments. An *in vivo* study was conducted to evaluate the effects of dietary polyphenol extracts (PE) from olive by-products—olive mill wastewaters (O) and olive leaves (L)—on the gut microbiota of broiler chickens. The PE were derived from Olea europaea cv. Carolea. Extracts from O were processed via spray- drying, yielding ~1 μm particles, while those from L were nano-encapsulated with chitosan. At 35 days of age, 54 male Ross 308 broilers were randomly assigned to three dietary groups (5 replicates/group, 6 birds/replicate): control (C) with a basal diet; O group with 400 mg/kg feed of total phenols; and L group with 272.3 mg/kg. Faecal samples were collected before (T0) and after 14 days (T1) of supplementation. DNA was extracted and analyzed using 16S rRNA gene sequencing to obtain microbiota profiles. Results showed significant β-diversity changes between T0 and T1. Both supplemented groups showed a reduction in species from the *Campylobacteraceae* family compared to the control. Notably, the O group had increased levels of Lactobacillus and reduced levels of *Enterococcus* and *Escherichia-Shigella* compared to the L group. In conclusion, supplementation with olive mill wastewater-derived PE positively modulated gut microbiota, promoting beneficial bacteria and reducing potential pathogens. These findings support the potential of olive-derived polyphenols, particularly from O, as natural and sustainable alternatives to conventional antimicrobials in poultry production.

The research activity was carried out within PRIN: PROGETTI DI RICERCA DI RILEVANTE INTERESSE NAZIONALE – Bando 2020, "Sustainable poultry production chain: biocontrol measures, new dietary approaches and novel packaging strategies", 2020ENLMHA_003, CUP E33C22000320001.

By-products from a "marginal" plant species, prickly pear, as additional ingredients to fermented milk with potential health and nutritional benefits

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The application of processing waste/by-products along with lactic acid bacteria could represent a possible strategy to confer functional aspects to food items. Prickly pear is a plant that grows even in marginal areas, with no input in terms of water, fertilization, etc…Seeds from prickly pear fruits represent by-products that are dried and milled to obtain a commercially available flour, that is rich in fiber, minerals and phenolics.

This study aimed to evaluate the impact on yogurt of incorporating prickly pear seed (PPS) flour, together with individual commercial strains of lactobacilli (*Lacticaseibacillus casei* BGP93, *Lacticaseibacillus casei* LC4P1, *Lacticaseibacillus rhamnosus* LRB, *Lactiplantibacillus plantarum* LPAL), some of which show probiotic traits. Preliminarily, we optimized the protocol by examining various percentages of PPS flour added either before or after fermentation. The best result was achieved adding 5% of PPS flour after fermentation, coinciding with the addition of lactobacilli. The ability of all lactobacilli strains to survive in the yogurt and in the presence of the starter cultures was assessed, during 40 days of refrigerated storage.

Adjunct lactobacilli and yogurt starters were quantified using both culture-dependent (plate count on MRS and M17 agar) and culture-independent (real-time qPCR) techniques. The inclusion of PPS flour resulted in elevated levels of fiber (more than 3%) and minerals, especially for magnesium, potassium and phosphorus. Furthermore, the antioxidant capacity, measured *in vitro* (via DPPH and FRAP assays), showed an increase in the yogurt added with PPS and *L. casei BGP93*. When the extracts of fermented milk beverages were tested in eukaryotic cell lines, antioxidant and anti-inflammatory activities were detectable. These findings suggest the suitability of integrating PPS flour and adjunct lactobacilli into yogurt formulations to obtain functional yogurt, leading to a greater availability of phenolic compounds. Further research is needed on consumer appreciation and willingness to pay.

Microbiomes, edible packaging, and quality evaluation: emerging trends fresh-cut products

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Packaging solutions play a vital role in limiting microbial growth, a key factor in maintaining the quality and extending the shelf life of fresh produce. Recent innovations suggest that microorganisms can be integrated into packaging systems, offering novel, sustainable strategies for food preservation. Lactic acid bacteria (LAB) and microbiomes (e.g. milk kefir grains) isolated from fermented foods have been incorporated into packaging materials (including edible packaging) demonstrating potential for biocontrol activity, spoilage reduction, and support of environmentally friendly food systems. Combining experimental results and literature information, we also evaluated the strengths and limitations of microbiome-based packaging compared to conventional approaches, within the context of two ongoing research projects: "INTelligent, ACTive MicroBIOme-based, biodegradable PACKaging for Mediterranean food (INTACTBioPack)", funded by the PRIMA Programme Section 2 (2023), and "ON Foods—Research and Innovation Network on Food and Nutrition Sustainability, Safety and Security (OnFoods)" (Project code PE00000003), funded by NextGeneration EU under the Mission 4, Component 2, Investment 1.3 initiative. Additionally, this review examines the role of hurdle technology and integrated solutions involving physical postharvest strategies, assessing their impact on the preservation of fresh-cut produce. Unlike conventional static methods, microbiome-based approaches offer dynamic, biodiversity-driven solutions for food preservation. In conclusion, this study highlights the transformative potential of microbiome-integrated packaging and biocontrol technologies in postharvest management. However, challenges remain, particularly regarding regulatory approval, microbial stability, and scalability. Future research should aim to optimize the compatibility of microbiomes with packaging materials, ensure controlled microbial activity, and assess consumer perception and acceptance.

Lactic acid bacteria for more efficient re-use of agro-food waste in the food industry

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The valorization of wastes and by-products through microbial-based biotechnological solutions for more efficient re-use in the food industry represents one of the crucial issues in the green transition of food systems. Using apple, orange, tomato, broccoli, white cabbage, carrot, potato, buttermilk, scum, spinning water, and whey as model matrices and a panel of ten different lactic acid bacteria (LAB) strains, we evaluated the combination of fast screening and digital solution as emerging approaches to optimize the management of the strains in the different matrices/conditions. For each of the matrices, after fermentation with a selected LAB strain, we proposed a case study of re-use in the food industry. In the framework of evaluating the feasibility of these optimization strategies, different physical, chemical and biological contaminants were studied to investigate the safety of the treated wastes/by-products.

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Microbiological safety and potential prebiotic activity of *Acheta domesticus* insect flour: a novel sustainable ingredient

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Entomophagy is associated with several nutritional, economic, social, and environmental benefits. However, scientific research is still needed to assess the potential safety risks linked to the consumption of edible insects, particularly from the perspective of regulators, markets, and consumers. In this context, the microbiota of cricket feeds and *Acheta domesticus* flour were analysed thorough investigation using culture- dependent approach. Additionally, cricket flour (10 g/L) was evaluated in monocultures of *Lacticaseibacillus paracasei, Lacticaseibacillus rhamnosus, Bifidobacterium animalis* and *Bifidobacterium longum* (at a final cell density of 10 CFU/mL), with the aim of exploring its potential as a functional food ingredient. A commercial fructooligosaccharide (FOS) was used as positive control, while a modified MRS broth without dextrose served as blank. At intervals of 0, 24, and 48 hours, strain growth was assessed through microbial plate counts. All monitored microbial groups were detected in the analysed feed samples. Notably, the highest microbial densities were observed for yeasts and molds, with counts of 5.33 and 5.51 Log CFU/g, and for mesophilic aerobic bacteria, with densities of 5.98 and 5.46 Log CFU/g in carrot and laying hen feed, respectively. In contrast, microbiological analyses of cricket flour revealed the absence of all targeted microbial groups, except for total aerobic mesophilic bacteria, which were detected at a low density of 2.09 Log CFU/g. Focusing on prebiotic activity, *Acheta domesticus* flour showed significant efficacy compared to the control, supporting high growth densities of all tested strains after 24 hours of incubation. In conclusion, this study contributed to the characterisation of the microbiota associated with edible insects to evaluate the influence of rearing and processing conditions on the microbial safety and prebiotic functionality of insect-based products.

The work was supported by the Ministry of University and Research (Next Generation EU - PNRR) TECH4YOU innovation ecosystem project DM 1049.

Polyphasic characterisation of lactic acid bacteria diversity associated with sheep milk from 'Gentile di Puglia': starter culture design for Pecorino cheese and for cereal-based fermented beverage

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Preserving regional agrobiodiversity is a fundamental strategy for promoting more sustainable food systems. Fermentation represents a family of bioprocesses that is interesting for achieving greater environmental, economic, and social sustainability. The synergistic added value that can be realized by combining the valorization of biodiversity and fermentation processes can be particularly relevant for sustainable development in marginal areas. In this work, we report on a case study of Apulian (Southern Italy) marginal areas (Subappennino Dauno), including animal, plant, and microbial resources. Among the region's agricultural heritage is the 'Gentile di Puglia' sheep breed, whose name, meaning "Gentle Apulian," refers to its fine wool but is also renowned for its milk used in cheese production. Starting from a 'lattinnesto' from raw milk, we (i) isolate lactic acid bacteria (LAB) from the milk of the 'Gentile di Puglia' sheep breed and (ii) perform a polyphasic characterization of these LAB strains to design a multi-strain starter culture for Pecorino cheese production. Furthermore, the same LAB strains were used to create a novel cross-over fermented cereal-based beverage using the 'Senatore Cappelli' variety of durum wheat typical of this area.

Mariagiovanna Fragasso, Giuseppe Spano, Vittorio Capozzi, and Hülya Cunedioglu are supported by the funding of the European Union Next-Generation EU PNRR Mission 4 Component 2, Investment 1.4 D.D. 1032 17 June 2022, CN00000022 within the Agritech National Research Centre for Agricultural Technologies.

Effects of High Pressure Processing (HPP) and thermization on the physicochemical and sensory characteristics of Fontina PDO cheese

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This study aims to evaluate the effects of High Pressure Processing (HPP) and thermization on the physicochemical and sensory characteristics of Fontina PDO cheese, a traditional semi-hard, smear-rind cheese produced in the Aosta Valley (Italy). The trial was conducted to assess the compliance of the treated cheese and to validate the methods in view of a potential revision of the Fontina PDO production specifications.

The study involved the application of thermization (64°C for 40 seconds) and HPP (at pressures exceeding 6000 bar) at different stages of the production process, followed by sensory evaluations. Specifically, both winter and summer cheese produced from raw milk were analyzed, as well as cheese treated with HPP at the end of the ripening period. Additionally, cheese produced from raw milk and thermized milk were examined. Sensory evaluation was performed by a trained panel according to ISO standards, with particular focus on appearance, aroma, taste, texture, and overall acceptability.

The sensory analysis showed no significant differences between the HPP-treated alpine cheese and the corresponding untreated samples, while the HPP-treated dairy cheese exhibited a slightly improved sensory profile. Similarly, no significant differences were observed between cheese made from raw and thermized milk.

Both HPP and thermization represent valid technological options for extending the shelf-life and ensuring the microbiological safety of Fontina PDO cheese, while affecting its physicochemical and sensory characteristics in different ways.

Effect of glutamate induction and oxygen on the production of γ-aminobutyric acid in *Levilactobacillus brevis* LB12

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γ-aminobutyric acid (GABA) is a non-proteogenic aminoacid that provides several physiological benefits to human health. Among lactic acid bacteria (LAB), many strains belonging to the species *Levilactobacillus brevis* have been recognised as potential GABA producers and have been investigated and characterized for their ability to produce GABA in synthetic media and/or food matrices. In this study, *Lvb. brevis* LB12 was used to evaluate the effect of glutamate induction and atmosphere of incubation (anaerobiosis vs aerobiosis) on biomass yield, GABA production, and expression of GAD operon genes. Glutamate (Glu) consumption and GABA accumulation in growth supernatants were estimated with Thin Layer Chromatography and densitometric spot analysis, while the relative expression of *gadB*/*gadA* (glutamate decarboxylase), *gadC* (glutamate: gamma-aminobutyrate antiporter), *gadR* (transcriptional regulator) and *gltX* (glutaminyl-tRNA synthetase) genes was evaluated by using RT-qPCR (SYBR Green protocol), in order to elucidate the activation and regulation mechanisms involved in GABA synthesis. AE conditions and Glu supplementation increased biomass yield in *Lvb. brevis* LB12, but the presence of oxygen impaired the efficiency of Glu to GABA biotransformation, resulting in a lower GABA accumulation. The expression of genes belonging to GAD operon (*gadB, gadC, gadR*) was also impaired in AE-growing cells. Our results confirmed that *gadB* and *gadC* are co-regulated and are under the control of *gadR* gene. *gadA*, instead, was affected to a lesser extent by atmosphere of incubation and Glu supplementation, confirming that *gadB* was mainly involved in Glu-GABA conversion. This work provides further insights on the factor affecting GABA production and regulation mechanisms of GAD operon, although additional investigations are needed to maximize the efficiency of Glu-GABA conversion in *Lvb. brevis* LB12.

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Scouting vinegar fermentation at laboratory scale as a tool to valorize organic fruits destined to waste

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This study focused on valorizing third-category fruits from organic farming, often destined to waste, through their fermentation to vinegar. For vinegar production at laboratory scale, juices from apples, peaches, and clementines collected in Apulia region (Italy) were standardized to 18 °Brix and underwent a sequential two- step fermentation process: alcoholic fermentation using *Saccharomyces cerevisiae* DSM 1848 (from beer) at 7 log CFU/ml, followed by acetic acid fermentation with *Gluconobacter oxydans* DSM 7145 (from beer) at 7 log CFU/ml. Alcoholic fermentation, at 15 °C, lasted 10 days and yielded ethanol concentrations from 5% (v/v), for apple and clementine juices to 5.94%, for peach juice. The secondary fermentation (at 15 °C, 20 days) produced acetic acid up to 5.61% (w/v), with peach vinegar (PV) showing the highest acidity. Total coliforms were below the detection limit throughout the process. The three experimental vinegars were subjected to descriptive sensory analysis, along with a commercial apple vinegar. Clementine vinegar (CV) achieved the highest overall acceptability (8.0 ± 0.7) due to balanced sweetness (6.3 ± 0.8) and citrus aroma (5.1 ± 0.9), while PV was scored with the highest color (9.5 ± 0.9) and aroma (10.7 ± 0.9).

Antioxidant activity was in vitro evaluated (DPPH and FRAP) on the experimental vinegars and the commercial one. CV exhibited a DPPH scavenging activity of 83.97% and a FRAP absorbance at 700 nm of 2.68 Units of Absorbance (UA), while PV reached 81.56% scavenging activity and 2.37 UA. Apple vinegar showed lower antioxidant activity (59.21% DPPH, 2.08 UA), and the commercial vinegar had the weakest activity (40.67% DPPH, 1.19 UA). The results highlight the potential of organic fruit matrices to yield high-quality, value-added organic vinegars with enhanced sensory and health-promoting attributes.

Further research could scale-up the process, in order to apply it to fruit destined to waste.

Session 3

Soil and Plant microbiota: rooting for sustainable agriculture

Plenary Lecture

From the ecology and functions of the plant microbiome to its importance in the food system

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Complex microbiota are found in association with all different plant tissues, from the below-ground organs like roots/rhizosphere to above-ground tissues and reproductive organs. Depending on the tissues, vegetation stage and many other environmental factors, microbial communities are differently composed, driven by different parameters and are also responsible for multiple holobiont functions. This talk will address the ecology and functioning of microbiota in different plant tissues, e.g., the ecology and functioning of seed microbiota, or the potential role of potato root and rhizosphere microbiota in regard to drought stress mitigation and linkage of microbiome and plant phenotypic traits. This talk will also address application-relevant aspects as well as the role of (plant) microbiomes in the food system.

Pyrroloquinoline quinone (PQQ)-producing *Arthrobacter sp*. D4 benefits peanut growth, induces antioxidant response and favourably modulates the rhizobacterial community: a prospective strategy towards antioxidant agriculture

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Plant rhizosphere displays multipartite interactions, involving plant growth promoting rhizobacteria (PGPR), known to be essential determinants of crop yield, soil health and rhizomicrobial functions. PGPR capable of boosting plant antioxidant system emerge as promising tools to develop oxidative stress resilience in plants. *Arthrobacter sp*. D4 (D4), isolated as a methylotrophic phosphate-solubilizer with siderophore and IAA-producing abilities; remarkably also produced pyrroloquinoline quinone (PQQ). Present work characterizes influence of D4 on *Arachis hypogaea* (peanut) growth, defense physiology and associated rhizobacterial community. Introduction of D4 as talc-bioformulation in peanut rhizosphere, enhanced root length and plant dry weight by ~35% and >95% respectively, 30 days post-inoculation in pot set-up. PQQ levels increased by ~57.6% and ~9% in treated peanut roots and shoots, respectively while carotenoid, chlorophyll, flavonoid and phenol content in leaves enhanced by ~6.54%, 12.7%, 68.1% and 161%, respectively. Increased activities (~2-5 fold) of antioxidant and stress-response enzymes like catalase, peroxidase, polyphenol oxidase, glucanase and phenylalanine lyase in roots, shoots and leaves of D4-treated peanut plants as compared to untreated control, suggested induced adaptive antioxidant response. Further, 16S rDNA-based metagenomic analysis estimated a significant distinction in the rhizobacterial diversity and abundance in response to D4 treatment, especially enriching the plant- beneficial genera like *Bacillus, Gaiella Rubrobacter* and *Streptomyces*. PICRUSt2 analysis predicted that while major metabolic and cellular functions of the rhizobacterial community remained unperturbed, 10% increase and ~7% decrease in abundance of polyketide biosynthesis and lipopolysaccharide biosynthesis proteins was noted, respectively. PQQ is largely recognized as multifunctional nutraceutical and plant growth promoter, with excellent antioxidant potential. Given that PQQ biosynthesis is limited to bacteria, PQQ-producing PGPR could help preventing photo-oxidative damage in plants and contribute to sustainable strategies towards the modern approach of 'antioxidant agriculture'.

Effects of municipal solid waste (MSW)-derived compost on the vineyard soil microbiota

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Application of municipal solid waste (MSW)-compost as a mulching material in vineyards supports sustainable practices and aligns with both circular economy and zero-waste principles. The aim of this study was to evaluate the effects of MSW-compost mulching on the vineyard bacterial soil microbiota structure and its correlations with the physico-chemical parameters. The experiment was conducted in a 5-years-old vineyard (cv. Primitivo) in Salento (Southern Italy). Vineyard implantation consists of rows, vertical shoot positioning and Guyot pruning. Grapevine plants were divided into groups of 6 plants, subjected to the following treatments (n=3, completely randomized design): Compost (22): mulching with MSW-compost, 22 kg/plant; Compost (15): mulching with MSW-compost, 15 kg/plant; Compost (8): mulching with MSW-compost, 8 kg/plant; Film-PE: mulching with traditional black plastic film; Film-Bio: mulching with biodegradable film; Control: no mulching.
MSW-compost consisted of a composted mixture of green residues and the organic fraction of municipal solid waste (MSW) produced by a local company. Metabarcoding was performed on Illumina Miseq2 (V3-V4 region of 16S rRNA gene), on samples collected in March, July and October 2023. Data were analyzed with QIIME2, to inspect α and β diversity, and assign taxonomy. Statistical comparison of taxa (genus-level) abundance between groups were done in STAMP. CoNet app was used for co-occurrence network analysis. No significant effects were observed on both alpha and beta diversity. However, compost-treated samples clearly segregated from control. Seventy-six out of the 135 most abundant taxa (>0.15%) collected in October, showed correlations with physico-chemical parameters; seven of them were enriched by compost compared to non-mulched control, including putative beneficial taxa, like *Flavobateriaceae* and *Sphingobacteriaceae*, that showed positive correlations with both organic carbon and nitrogen concentration, and that were also main hub taxa in the co- occurrence network.

Selection and evaluation of salt-tolerant plant growth promoting bacteria from halophyte plants for sustainable agriculture

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Soil salinity is one of the most significant abiotic stresses limiting agricultural productivity worldwide. Nevertheless, saline environments are home to diverse microbial communities, many of which have unique genetic and physiological adaptations that allow them to survive and thrive in high-salinity conditions.

Among these, Plant Growth Promoting Bacteria (PGPB) associated with halophytic plants, both in the rhizosphere and endosphere, may play a crucial role in enhancing plant health and resilience by improving the availability and uptake of essential nutrients such as carbon, nitrogen, and minerals. Isolating and characterizing halotolerant or halophilic PGPB is a sustainable way to increase crop yields in saline soils. Microbial communities adapted to such conditions have been identified in various saline environments. A notable example is the Margherita di Savoia saltworks in north-eastern Apulia, Italy, which harbours a distinctive and largely unexplored indigenous microbiota.

This study aimed to isolate and characterize potential PGPB from two halophytic species, sea rocket (*Cakile maritima* Scop.) and saltwort (*Salicornia* spp.), collected from two sites in the Margherita di Savoia area. Microbiological sampling was conducted in triplicate during three phenological stages (seedling, vegetative, and flowering) in 2023. In total, 290 bacterial strains were isolated and characterized using morphological, biochemical, and molecular methods; 250 were rhizobacteria and 40 were endophytes.

Selected halotolerant isolates exhibited potential plant growth-promoting traits, including phosphate and silicon solubilization, indole-3-acetic acid (IAA), siderophores, and ammonia production, and tolerance to salt stress. Three strains with the most promising traits, identified genotypically, were tested in a growth chamber on *C. maritima* and cultivated lettuce (*Lactuca sativa*) under high salinity. Results showed significantly enhanced plant growth, especially under moderate salinity, compared to non-inoculated controls.

A simplified holobiont system revealed *Rhizobium* sp. GR12 as a possible versatile biofertilizer in grapevine under different abiotic stresses.

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The success of Plant Growth Promoting Bacteria (PGPB) as biofertilizers is still hampered by a limited understanding of their establishment in the receiving ecosystem. Therefore, it is pivotal to go beyond the study of the plant-microbiome-PGPB interplays in the model plant *A. thaliana*. Here, we used micropropagated grapevine plantlets obtained via somatic embryogenesis to disentangle the effect of potential PGPB under different abiotic stresses. Under *in vitro* conditions, we tested *Rhizobium sp*. GR12 and *Kosakonia sp*. VR04 showing by qPCR that both strains effectively colonized the plant endosphere. However, only the inoculation with *Rhizobium sp*. GR12 increased root biomass when plantlets were subjected to nutritional deficit. Using high-throughput sequencing and culturomics, we described the bacterial community of micropropagated grapevines, revealing the shifts of composition, diversity and microbial interactions in response to the PGPB invasion. The data showed that *Rhizobium sp*. GR12 had a minor impact on the recipient endophytic community and preserved its structure despite the effective colonization, highlighting how plant-endophyte associations rule the holobiont performance. We further tested this strain on potted micropropagated vines exposed to water deficit. *Rhizobium sp.* GR12 remained viable in the rhizosphere of both water stressed and irrigated plants. Its inoculation significantly promoted the plant height and root system growth. Physiological and molecular responses were also investigated, showing that, under water stress, *Rhizobium sp.* GR12 improved the plant water use efficiency, increased the concentrations of abscisic acid, jasmonic acid and viniferin in the root and the proline content in the leaf. Moreover, both in leaves and roots, GR12 inoculation enhanced the transcription of the dehydrin gene VvDH, prompting the endogenous defence machinery of grapevine in response to water deprivation. Our approach indicated *Rhizobium sp*. GR12 as a possible versatile biofertilizer under abiotic stress scenario, providing a novel system for holobiont studies of interest in agriculture.

Enhancing agricultural sustainability: selection of *Rhizobium sullae* strains for optimized nitrogen fixation from populations of *Sulla coronaria* in Sicily

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Agricultural systems are essential for fulfilling human needs; however, they account for approximately one- third of anthropogenic greenhouse gas emissions and contribute to environmental challenges such as biodiversity loss, soil fertility depletion, and the release of toxic substances into ecosystems. The use of legumes, such as *Sulla coronaria*, offers a promising avenue for enhancing sustainability due to its ability to fix atmospheric nitrogen in symbiosis with specific microorganisms. *Sulla coronaria* is a forage legume with broad distribution in the Mediterranean regions, renowned for its adaptability and capacity to produce high- quality forage. Its moderate content of condensed tannins can positively affect ruminant health and mitigate enteric methane emissions. Furthermore, its root system improves soil chemical and physical properties.

Sulla coronaria can be used in crop rotations with cereals (especially in organic systems), for soil protection and restoration, honey production, and landscaping. However, the spread of *S. coronaria* is heavily reliant on its specific microsymbiont, *Rhizobium sullae*, which is essential for efficient nitrogen fixation through effective nodulation. This study aims to select various *R. sullae* strains with optimal nitrogen fixation and nodulation performance to develop a commercial inoculum. To achieve this goal, root nodule samples of *S. coronaria* were collected from 20 sites across six provinces in Sicily. A total of 151 bacteria were isolated and tested using species-specific PCR; 34 were identified as *R. sullae*, with their identity confirmed by 16S rRNA sequencing. RAPD-PCR was then applied to differentiate among strains, resulting in the identification of distinct genetic clusters. The study is currently in progress: strains selected from each genetic cluster are being inoculated into pots containing *S. coronaria* plants and cultivated in a controlled climatic chamber to assess their efficiency in nodulation and nitrogen fixation.

Monitoring of mobile genetic elements associated with AMR and VF in microbial biostimulants: a safety assessment based on *in silico* data and scientific literature

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Microbial biostimulants (MBs) contribute significantly to sustainable agriculture by promoting plant growth and reducing the need for chemical inputs. As their commercial use increases, including taxa not listed under the Qualified Presumption of Safety (QPS), there is an increasing need for systematic safety evaluations that can also enrich our understanding of these beneficial microbes. In this study, we applied an integrated genome-wide framework to assess the safety of commonly used bacterial MBs. Three QPS species (*Bacillus velezensis, Priestia megaterium, Rhodopseudomonas palustris*) and four non-QPS genera (*Azotobacter, Azospirillum, Herbaspirillum, Arthrobacter*) were selected based on their documented plant-associated ecological roles, including nutrient acquisition, phytohormone modulation, stress tolerance, and nitrogen fixation. Taxonomic accuracy was ensured combining Average Nucleotide Identity (ANI), digital DNA–DNA hybridization (dDDH), and single-copy core gene phylogeny. This analysis found misassignments in public databases, particularly within *Azospirillum* and *Arthrobacter* taxa, emphasizing the importance of taxonomic resolution in risk assessment. A curated set of 1,634 genomes was then screened for antimicrobial resistance (AMR), virulence factors (VFs), and mobile genetic elements (MGEs). As expected, QPS taxa showed minimal genomic risk, with only intrinsic, non-transferable AMR features. Among non-QPS genera, Azotobacter carried VFs related to environmental adaptation rather than pathogenicity, while conserved chromosomal β-lactamase-like genes were identified in *Azospirillum* and *Herbaspirillum* without evidence of mobile flanking regions. Phenotypic validation via MIC assays confirmed low-level resistance in *Azospirillum*, consistent with intrinsic traits and literature reports. Overall, our findings indicate that the application of these taxa as MBs does not raise immediate safety concerns. Importantly, the integrated genomic-literature approach proved highly effective not only in validating safety but also in uncovering novel genomic features relevant to rhizocompetence and ecological performance. This dual benefit makes the approach valuable for both regulatory assessments and the future development of microbial biostimulants.

Unravelling the endophytic bacterial microbiota of tomato roots: a step towards sustainable agriculture

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Plant associated microbiome is a resource of genetic and microbial biodiversity which can be harnessed for biotechnological applications. Assessing the microbiome dynamics across different locations, crop managements, and plant growth stages may lead to powerful insights into microbial community behaviour, making it possible to develop precise biotechnologies. Native microbiota, and particularly endophytic one, is naturally adapted to the host plant, showing persistence throughout plant life cycle and enhancing plant growth. In this study an *in silico* approach based on a large-scale analysis of tomato roots endophytic prokaryotic community was proposed, using 16S amplicon data from 192 plant roots samples. Two main tomato types were sampled in two Italian regions, considering all the cultivation conditions normally used for tomato crops. Phyla-level analysis revealed that the microbial ecosystem followed precise patterns in the two sampling regions, where Actinobacteriota and Proteobacteria dominated, while less represented phyla were Bacteroidota, Verrucomicrobiota, Patescibacteria and Firmicutes. A more in-depth analysis at low taxonomic level allowed us to uncover transient (discriminant analysis among time points) and persistent (core microbiota analysis) host-microbe relationships. *Streptomyces*, *Shinella*, *Devosia*, *Pseudoxanthomonas*, *Pseudarthrobacter*, *Lechevalieria*, *Bosea*, as well as *Variovorax paradoxus*, and *Sphingobium herbicidovorans*, were among the most important components of endophytic prokaryotic microbiota of tomato roots. This work gives a characterization of the microbiota associated to tomato roots and provides a strong basis for developing tailored microbial formulations, aimed at developing eco-friendly strategies for crop management.

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Genetic and functional diversity of arbuscular mycorrhizal fungal spore associated bacteria occurring in Mediterranean sand dunes

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Arbuscular mycorrhizal fungi (AMF) are beneficial soil microorganisms that establish mutualistic symbioses with the roots of most plants. They promote plant growth, nutrition and tolerance to both biotic and abiotic stresses. The key functions of AMF are facilitated and complemented by bacterial communities living associated with their hyphae and spores, and playing important roles as plant growth promoting and mycorrhiza helper bacteria. This study aimed at molecularly and functionally characterizing the bacterial communities strictly associated with AMF spores occurring in the rhizosphere of *Ammophila arenaria*, a xerophytic plant inhabiting maritime sand dunes, a nutrient-poor and harsh environment. High-throughput Illumina sequencing was used to analyse the bacterial communities associated with the two dominant AMF species, *Racocetra fulgida* and *Racocetra persica*. Notably, two endosymbionts, Ca. *Moeniiplasma glomeromycotorum* and Ca. *Glomeribacter gigasporarum*, differentially occurred in the two AMF species, together with highly diversified bacterial communities affiliated to 69 and 34 genera in *R. persica* and *R. fulgida*, respectively (251 Amplicon Sequence Variants). Most of the identified bacteria belonged to culturable genera and species, reported to possess key beneficial activities. In order to analyse the functional diversity of such bacterial communities we isolated a total of 203 and 81 bacterial strains from *R. persica* and *R. fulgida* spores, respectively. Their ability to produce extracellular polymeric substances (EPS), key compounds that enhance water retention and protect roots from desiccation, was assessed. 91 strains identified as high EPS producers were subsequently screened for salinity tolerance. Among them, 13 isolates were able to grow in the presence of up to 10% NaCl, 10 of which also produced 1-aminocyclopropane-1- carboxylate deaminase and indole-3-acetic acid. Such bacterial isolates will be further tested *in vivo* to identify the best performing ones to be used in the formulation of effective microbial consortia, able to enhance plant productivity and resilience in harsh agroecosystems.

Exploring the potential of lactic acid fermentation to produce innovative biostimulants

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Climate change is intensifying extreme events such as drought and excessive rainfall, impacting crop productivity and fruit quality. Tomato (*Solanum lycopersicum* L.), one of the most widely cultivated vegetables worldwide, is susceptible to water availability. Enhancing the sustainability of the agricultural sector has focused on the use of biostimulants, natural products shown to improve various aspects of plant growth, yield, nutrient use efficiency, and resistance to both biotic and abiotic stresses. In recent years, a wide range of biostimulants has been developed from diverse sources, including microorganisms and agro-industrial by-products. The amount of agricultural and urban waste is increasing in line with the growth in the global population and food demand. Consequently, research has turned toward the reuse of food waste and by-products through fermentation, an environmentally friendly strategy for valorizing such materials. Lactic acid bacteria have attracted attention for their promising application as agricultural biostimulants. This two-year study investigated the effects of innovative biostimulants produced via lactic acid fermentation of discarded kiwi and pumpkin biomass on the yield and quality of tomato plants, grown in greenhouses and open fields under varying irrigation conditions: water deficit, optimal irrigation, and water excess. Biostimulants were applied either as foliar sprays or soil inoculants. The harvested fruits were analyzed for morphological traits and chemical composition. Under water-deficit conditions, biostimulant treatments proved promising in terms of both the physiological response and productivity of the tomato plants, particularly when compared to optimally irrigated plants. Overall, the beneficial effects of these treatments were more pronounced under abiotic stress, suggesting that such products can effectively mitigate the negative impact of water stress. This highlights the potential of agro-industrial by-products within a circular economy framework, in which a by-product can be transformed into a valuable tool to reduce the effect of climate change and promote more sustainable agriculture.

Characterization of stable poultry manure: microbial composition and effects on crop growth and yield

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Organic amendments from the aerobic transformation of livestock manure enhance soil fertility and carbon storage. Poultry farming, especially in Europe, produces large amounts of manure. Its biological stabilization not only manages waste but can also yield a product containing beneficial, plant growth-promoting bacteria. This study, in collaboration with FOMET S.p.A. investigated the predominant native microbial components of stable poultry manure. Samples were collected over two years under identical conditions at three stages: the start and end of aerobic fermentation, and after pelleting. Chemical and microbiological analyses revealed a significant decrease in pH and moisture, alongside an increase in total nitrogen, primarily from organic sources. Bacterial colonies were isolated from the final product on TSA plates. From the final product, 45 bacterial colonies were isolated (respectively, in the two sampling years), with 27 strains identified as Bacillus spp., likely due to their heat-resistant endospores. The plant growth-promoting characteristics of selected Bacillus strains were then investigated through *in vitro* assays and Biolog phenotyping.

Moreover, plant growth assays were performed on two horticultural crops, lettuce and eggplant, showing a significant increase in fresh weight in the lettuce and an increase in eggplant productivity when poultry manure was added to the substrate, in comparison with the respective controls. Total bacteria content analyses were performed by qPCR at the beginning and end of the experiment, showing a significant increase in bacteria in the treatments where the manure was applied.

The present study demonstrates the significant impact of stable poultry manure on plant productivity, which can be partly attributed to the presence of valuable microbial components. Next-generation sequencing analyses are being conducted to study the evolution of microbial and fungal composition during manure fermentation. Additionally, tunnel experiments on tomatoes, cabbages and onions are investigating crop productivity and the chemical and microbial status of the soil.

Harnessing native Bacillus sp. for sustainable enhancement of saffron and other vegetatively propagated crops: A microbial strategy for ecological transition

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Crocus sativus L, saffron, is the world's most valuable spice, containing bioactive compounds—crocin, picrocrocin, and safranal— is widely used in medicinal, cosmetic, perfumery, and textile industries.

However, in the saffron-growing region of Jammu and Kashmir, India, cultivation has seen a significant decline, primarily due to low cultivation area and corm rot disease caused by Fusarium oxysporum. The indiscriminate use of chemical fungicides and fertilizers to overcome such challenges has negatively impacted soil health and beneficial microbial communities. To address these issues, our research group isolated a native Bacillus sp. strain D5 (Bar D5) from saffron corms, exhibiting strong plant growth- promoting (PGP) traits and in vitro antagonism against *Fusarium oxysporum* R1 (Fox R1). A bioformulation developed using this strain was extensively tested for nine years of field trials in saffron cultivation. Results demonstrated a two-fold increase in flower and stigma yield, a significant enhancement in the content of bioactive compounds (crocin, picrocrocin, safranal), and an improvement in soil health, evidenced by increased nitrogen, phosphorus, and potassium (NPK) levels. Following its success in saffron, the Bar D5 bioformulation was applied to other vegetatively propagated crops. In garlic, three years of field trials showed increased yield and allicin content—an organosulfur compound with antimicrobial and cardioprotective properties. In potato, two-year field data revealed enhanced tuber production and a reduction in reducing sugars, improving the processing quality of the crop. This study highlights the potential of native microbial resources in promoting sustainable agriculture. The Bar D5-based bioformulation offers an eco-friendly, scalable alternative to chemical inputs and is ready for technology transfer with proven applicability in saffron, garlic, and potato cultivation.

Dynamics and growth-promoting potential of rhizobacteria in wheat: a stage-wise evaluation of rhizosphere communities

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Rhizospheric bacteria, particularly plant growth-promoting rhizobacteria (PGPR), are key drivers of sustainable agriculture, as they enhance plant development even under environmental stress. However, the effective application of PGPR is often limited by the complex and dynamic nature of microbial interactions within the soil rhizosphere. Understanding how environmental and agronomic factors shape these microbial communities is essential for harnessing their potential in ecological transition strategies.

This study investigates the influence of cropping history and crop phenology on rhizospheric microbial diversity and the functional performance of plant growth-promoting rhizobacteria (PGPR) in spring wheat (*Triticum aestivum* L.). Fieldwork was conducted at Brackenhurst Farm, Nottingham, United Kingdom, comparing the rhizospheric soils of spring wheat cultivated in two fields with distinct long-term management histories: one under continuous cereal-legume rotation and the other incorporating intermittent periods of herbaceous grassland for grazing. For a temporal assessment of bacterial community dynamics, rhizosphere samples were collected at three key phenological stages: tillering, stem elongation, and spike formation.

Shifts in rhizospheric bacterial community composition were analyzed, and gene abundance was quantified to assess the impact of plant developmental stages and soil management practices on PGPR diversity and functional activity. In addition, bacterial strains were isolated and screened for their growth-promoting potential and drought tolerance, according to the specific growth stages. Sequencing results revealed significant differences in bacterial diversity across the three phenological stages and between the two field management systems. By elucidating the interactions among microbial diversity, plant phenology, and agroecosystem management, this study supports the development of context-specific PGPR inoculants tailored to crop requirements, environmental conditions, and soil characteristics, contributing to the advancement of resilient and sustainable agricultural practices.

Assessing the impact of plant-based amendments from aromatic plants on soil fertility and microbial diversity

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Sustainable agriculture is gaining increasing attention as a response to the environmental challenges posed by conventional farming systems. In this context, circular economy approaches offer promising solutions by transforming agricultural by-products into valuable resources. The processing of medicinal and aromatic plants (MAPs) generates residual biomass, known as exhausted herbs (EHs), which may serve as a sustainable organic amendment for soils. This project explores the potential of EHs to improve soil fertility and influence soil microbial community shifts. A key focus is to understand how EHs application affects the recruitment of root-associated microbial consortia that support plant growth, nutrient uptake, stress tolerance and pathogen resistance. A two-year field experiment was conducted in collaboration with a farmer cultivating MAPs and traditional maize varieties in Northern Italy. EH-based amendments were applied annually after the characterization of raw material, and soil and root samples were collected at the beginning and end of each maize growing season. To date, physical-chemical analyses and DNA extractions have been completed on 150 soil samples and 85 root samples. Ongoing and upcoming activities include morphological assessment of mycorrhization rate, high-throughput DNA sequencing (metabarcoding), and bioinformatic analyses to profile potential microbial community shifts. Additionally, a greenhouse trial is being planned to further investigate the influence of EHs under controlled conditions, offering deeper insights into the effects on soil microbiota dynamics and the potential agronomic benefits.

Sunflower at work: exploitation of the plant "cry-for-help" to recruit a degrading microbiome for soil rhizoremediation

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In polluted soils, positive plant-bacteria interactions may promote a Darwinian selection of holobionts that adapt as a whole to thrive in the presence of the contaminant. The aim of the work was to engineer the sunflower microbiome to obtain plant holobionts with the potential for a rhizoremediation intervention in a hydrocarbon-contaminated soil. Rhizoremediation relies on the biostimulation of the soil microbiome by plants, which drive in their rhizosphere the assembly and functionality of a microbial community enriched in degraders, in turn alleviating soil phytotoxicity. The rhizosphere microbiome is mainly recruited by the root exudates, which have been shown to change in stress conditions as a "cry-for-help" of beneficial microorganisms. Untargeted metabolomics demonstrated that sunflower shifts its root exudation pattern when exposed *in vitro* to xylene or was cultivated in the contaminated soil. Some of the metabolites elicited under pollutant stress were shown to improve the growth of selected bacterial hydrocarbon degraders. These findings provide the conceptual basis for the second part of the study, in which we exploited the sunflower's ability to "cry-for-help" of hydrocarbon-degrading microorganisms present in historically contaminated soil. We established indeed a collection of hydrocarbon-degrading bacteria from the rhizosphere of sunflower seedlings grown in this soil, which exhibited high phylogenetic diversity and comprised strains able to grow on several hydrocarbons as a unique carbon source. SynComs obtained from selected isolates, when inoculated on sunflower plantlets, were shown to promote plant growth in the contaminated phytotoxic soil. The impact of different "invading" bacterial inoculants has been, moreover, evaluated on the structure and degrading potential of the rhizosphere community by 16S rRNA amplicon sequencing and qPCR of degrading genes, showing strain-specific microbiome engineering effects. The results allowed obtaining harnessed sunflower holobionts through hydrocarbon-degrading strains and SynComs, paving the way towards validation in field experimental trials.

Controlling different *Fusarium* diseases in durum wheat with beneficial bacteria and their antifungal secondary metabolites

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Fusarium Crown and Root Rot (FCR/FRR) and *Fusarium* Head Blight (FHB), primarily caused by *Fusarium culmorum* and *F. graminearum*, are major threats to global durum wheat production, especially in organic systems where chemical control is limited. This study explores biological control agents (BCAs) as sustainable alternatives. Promising candidates, mainly from the *Bacillus* and Lactic Acid Bacteria (LAB) genera, were identified through *in vitro* screening. LAB strains reduced FCR/FRR symptoms caused by *F. culmorum* (Fc1126) and *F. graminearum* (Fg566) by 44.4% and 49.2%, respectively, in growth chamber trials. Two years of field trials in Northern Italy evaluated selected BCAs under natural conditions. In the first trial, seed treatments with *Bacillus amyloliquefaciens* CAAd improved seedling emergence by over 15% in both years. In the second, targeting FHB at the spike stage, spray application of *Apilactobacillus kunkeei* achieved the lowest FHB index (13.8) in the first year. In the second year, its cell-free supernatant (CFS) reduced the FHB index (4.2) compared to the inoculated control (7.6), suggesting an effect driven by antifungal secondary metabolites. Whole-genome sequencing of selected strains revealed genes associated with antimicrobial metabolite biosynthesis, which were further confirmed through metabolomic profiling.
Identified antifungal compounds included surfactin and fengycin (from *Bacillus*), and benzoic, phenyllactic, and hydrocinnamic acids (from LAB). To support scalable, low-cost BCA production, tomato peel, a metabolite-rich by-product of tomato processing, was tested as a fermentation substrate. *B. amyloliquefaciens* CAAd grew well in all peel-based media, with the highest OD600 at 24 h observed using dried, finely ground skin with Tryptic Soy broth. *A. kunkeei* showed strong initial growth in fresh unfiltered peel with De Man, Rogosa, and Sharpe broth, which declined by 48 h, likely due to nutrient depletion. This study highlights the potential of combining targeted BCAs with agro-industrial waste to sustainably manage *Fusarium* diseases in durum wheat.

Dark septate endophytes on native plants in arid and saline environments in the province of Almeria: diversity and evaluation of their pathogenic potential

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Agriculture is facing major challenges due to climate change, which negatively impacts crop yields. Among the most prominent effects are drought and salinity, which cause abiotic and biotic stresses on plants. To mitigate these effects, the use of beneficial microorganisms has been proposed, among which dark septate endophytic fungi (DSE) stand out. These fungi are not host-specific, as they are associated with a wide variety of plant species and habitats, suggesting a key role in plant survival and adaptation to extreme environmental conditions. Their identification in culture media is complex and therefore mainly relies on molecular tools. Most of the DSE present in soil belong to saprotrophic, mutualistic or pathogenic species of the orders *Helotiales, Chaetothyriales, Pleosporales, Capnodiales, Chaetosphaeriales* and *Sordariales.* The aim of this study was to determine the diversity of DSE in arid and saline environments in the province of Almería, and to evaluate its possible pathogenicity by artificial inoculation. Roots were collected from five different areas, sterilised and sown on PDA medium. Emerging hyphae were transferred to new media to obtain pure cultures. Molecular identification was performed by Sanger sequencing of the ITS1 and ITS4 regions, comparing the sequences obtained with the GenBank database using BLAST. A total of 415 isolates were obtained from 68 plants, from which 52 isolates belonging mainly to the phyla *Ascomycota* and *Phytiaceae* were selected. Among them, 29 isolates from eight different families were selected for pathogenicity tests on pepper and bean. The results showed an absence of pathogenic symptoms; on the contrary, an increase in aerial and root biomass was observed in some species. These results show the great richness of endophytic fungi in arid and saline ecosystems, as well as their potential as biostimulant or biological control agents, which makes them a promising tool for a more sustainable agriculture under adverse climatic conditions.

Optimization of large-scale production of Dark Septate Endophytic (DSE) Fungi

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Modern agriculture's increasing reliance on chemical inputs poses environmental and economic challenges, driving the search for sustainable alternatives. In this context, dark septate endophytic (DSE) fungi have emerged as promising bioinoculants due to their potential to enhance plant health and agroecosystem resilience. This study aimed to optimize the large-scale production of selected DSE isolates using low-cost solid substrates composed of varying proportions of sorghum, rice, salt (NaCl), and peptone.

Six fungal isolates were obtained from the roots of wild plants naturally adapted to arid, semi-arid, and saline environments in coastal Almería (Spain). Twenty-four substrate combinations were tested to promote the development of resistance structures (microsclerotia and spores), excluding cultures that only showed vegetative mycelial growth. Quantification of colony-forming units (CFUs) and sporulation was performed using a Neubauer chamber.

Isolates BC12, BC17, and BC27 —identified as belonging to the genera *Macrophomina*, *Sordaria*, and *Torula*, respectively— exhibited significant structural development under specific conditions. The highest spore concentration was recorded for isolate BC27, reaching 9.6×10^6 spores/mL in the treatment containing 90% sorghum, 10% rice, and 5 g of NaCl. Although all isolates displayed some salt tolerance, elevated concentrations negatively affected structural formation in most strains.

The solid formulation based on sorghum and rice effectively supported the development of resistance structures in several DSE isolates, representing a key step toward their industrial-scale production as bioinoculants. This study highlights the importance of adapting cultivation conditions to the physiological traits of each isolate to ensure viability, stability, and effectiveness in sustainable agricultural applications.

Efficacy of soil temperature modulation and *Trichoderma virens* as biological control against *Ganoderma boninense*-induced basal stem rot in oil palm seedlings

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Ganoderma boninense causes basal stem rot disease in oil palms by producing lignocellulosic enzymes that degrade plant cell walls, resulting in up to 80% yield reduction and approximately $28.4 billion USD in losses. This study evaluated an integrated management approach combining soil solarization, biocontrol, and chemical treatments under greenhouse conditions. Using a randomized complete block design with four treatments, soil temperature was elevated through solarization (reaching 43.7°C compared to 36.1°C in controls), followed by application of optimized levels of boron (0.2 mg/mL), potassium (35 mg/mL), and mancozeb (1 mg/mL) before planting. Golden Tenera oil palm seedlings were then subjected to four treatments: control (C), *Ganoderma boninense* K4/2 inoculation (G), Trichoderma virens K1-02 treatment (T), and combined *Ganoderma*+*Trichoderma* treatment (GT). After 12 weeks, lignocellulose content was significantly lower in the *Ganoderma* treatment (58.1±1.0%) compared to control (65.9±2.1%), *Trichoderma* (69.0±1.8%), and *Ganoderma*+*Trichoderma* (68.7±3.7%) treatments. PCR detection using *Ganoderma*- specific primers (Gan1/Gan2) and gel electrophoresis revealed ~200 bp DNA bands in roots from G and GT treatments at 20 weeks post-inoculation, confirming pathogen presence, while no *Ganoderma* DNA was detected in C and T treatments. Similarly, soil samples (5-10 cm from inoculation point) showed *Ganoderma* DNA only in G treatment, but not in GT, T, or C treatments. These results demonstrate that the integrated approach effectively inhibited lignocellulose-degrading enzymes from *G. boninense* K4/2, while T. virens K1-02 successfully prevented pathogen proliferation in both plant tissues and surrounding soil. This study confirms the successful translation of laboratory findings to greenhouse conditions for mitigating Ganoderma infection in oil palms.

Plant growth-promoting yeasts (PGPYs): a long-term sustainable solution for advancing climate-smart agriculture

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The use of microbial inoculants that beneficially interact with plants is a key pillar of climate-smart agriculture, aiming to reduce chemical inputs, enhance crop productivity and improve tolerance to abiotic stresses. This implies efforts to optimize the criteria for selecting potential plant growth promoters (PGPs), focusing also on yeasts, only recently investigated for their PGP potential. This study investigates the plant growth-promoting (PGP) properties of selected *Ascomycetous* and *Basidiomycetous* yeasts on zucchini (*Cucurbita pepo L.*), a fast-growing crop of high economic relevance. Yeasts were inoculated alone and in consortia to assess their impact on early stages of plant growth and their ability to mitigate salt-induced stress. Seed inoculation boosted the early phase of growth of the zucchini plants, primarily affecting root development. Three strains belonging to the species *Schwanniomyces etchellsii*, *Zygotorulaspora florentina* and *Holtermanniella festucosa* induced substantial increase in biomass and length of both epi- and hypogeal parts of the plant. Three other strains belonging to *Naganishia uzbekistanensis*, *Papiliotrema terrestris* and *Solicoccozyma phenolica* species, mitigated the growth inhibition caused by NaCl. Yeast inoculation also triggered strain-specific alterations in the biochemical profile of rhizosphere soil, suggesting active root-microbe interactions. Furthermore, the addition of PGPYs significantly altered the rhizosphere bacterial community and enriched the fungal community with the introduced yeasts. These findings underscore the potential of yeasts as promising, yet underexplored, PGP agents for sustainable agriculture under both optimal and stress conditions.

Root-associated microbial diversity as a tool to improve durum wheat resilience and agronomic performance

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Durum wheat (*Triticum turgidum* L. var. *durum*) represents a major food crop in Mediterranean and semi- arid environments, where its productivity is increasingly affected by drought and climate instability. To enhance resilience and maintain yields, microbial-based strategies are emerging as promising solutions. In this study, two cultivars of durum wheat, 'Svevo' and 'Puro', were inoculated with selected plant growth- promoting rhizobacteria (PGPR)—*Paraburkholderia graminis* and two *Bacillus* spp. isolates, respectively—and arbuscular mycorrhizal fungi (AMF)—*Rhizophagus irregularis* and *Funneliformis mosseae*, correspondingly—applied alone and in combination. 'Svevo' was grown in semi-controlled conditions under both irrigated and drought regimes, and physiological parameters such as daily transpiration and water-use efficiency were continuously monitored via a Plant Array Phenotyping Platform until the end of tillering. Root samples were then analyzed for mycorrhization rate. Plants grown to maturity were evaluated for aboveground biomass, thousand kernel weight (TKW), and total grain yield per pot. 'Puro' was tested in open-field conditions and analyzed for yield traits and the presence of *Fusarium*-associated mycotoxins, including deoxynivalenol (DON). In 'Svevo', all treatments except PGPR alone improved physiological performance and biomass accumulation. AMF and PGPR individually increased TKW and yield, with PGPR showing notable effects on reproductive output. Co-inoculated plants exhibited the highest AMF colonization, particularly under irrigation condition. In the open field experiment, inoculation with AMF enhanced total grain yield in 'Puro', while the PGPR–AMF combination significantly lowered DON content, indicating improved disease suppression. Results emphasized the distinct yet complementary benefits of PGPR and AMF under different growing conditions. Moreover, *P. graminis* may act as a mycorrhiza helper bacterium (MHB), promoting enhanced AMF colonization. The study highlighted the role of microbial diversity management as a promising tool to improve wheat resilience to biotic and abiotic stress, and to promote sustainable crop production under climate change scenarios.

Leveraging shot-gun metagenomics and correlation network analysis to investigate how interactions underpin microbial ecosystem function in soil

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Some of the most diverse microbial communities can be found in soils, and they play critical roles in nutrient cycling, carbon storage and food production. Microorganisms in soil share the same habitats and are likely to interact with each other frequently, however, the implications of microbial interactions to soil ecosystem function remain poorly understood. Enhancing our understanding of the relationships between microorganisms will improve our ability to predict and manage soil responses to disturbances. Microbial interactions in complex ecosystems are increasingly being studied using correlation network analysis, which can reveal information about potential interactions and shed light on how different microbial groups compete for similar niche spaces. Correlation network analysis is often carried out using microbial abundance profiles generated from high-throughput DNA sequencing of taxonomic marker genes, such as the prokaryotic 16S rRNA or the fungal ITS1 genes. This approach lacks functional information that can reveal how the metabolic properties of interacting microorganisms mediate interactions. In this study, 183 shotgun metagenomes were obtained from three paired pasture-woodland plots in South-Eastern Australia over a seasonal cycle. In addition, soil nutrient data were also collected at each time point. We annotated the metagenomes using a read-based approach and carried out network analyses of the sequences aggregated at the genus level. This approach has the benefit of reducing the loss of sequence data typical of assembly- based metagenomic approaches. Focusing on the strongest interactions, we investigated the functional annotation of sequences from interacting groups of microorganisms. We highlight the advantages of this approach compared to taxonomic marker-gene-based network analysis in revealing possible metabolic complementarities between interacting taxa and their links to ecosystem function.

The HEMUA project: a way to improve research, innovation and contacts with the labour market in the higher education system in Uzbekistan

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HEMUA (*Higher Education Modernization to boost Uzbekistan Agricultural system and promote excellence and regional development*) is an Erasmus+ CBHE project. The partnership is composed of two EU and four Uzbek academic partners plus an Uzbek association of agri-food companies. HEMUA follows up a series of initiatives that link the DISTAL (UNIBO) to the Samarkand State University in Uzbekistan.

The project was born after considering that the Uzbekistan higher education (HE) system presents some gaps related to the scarce connection between teaching and the rapid job market evolution. On the other hand, agriculture is undergoing a modernization process in Uzbekistan, and the HE system is not prepared to this transition yet.

The general objective of HEMUA is to prepare new professionals able to respond to the needs of modern agriculture by introducing in the Uzbek HE system upgraded teaching content and new teaching methods. One pilot course for each of the 4 Uzbek HE partners will be selected in the topics of: sustainable horticulture; agroecology; food safety and precision agriculture. The pilot courses will be modified according to the Bologna process model, students will receive English training, spend traineeships in companies in Uzbekistan and EU to gain work experience, and participate in the quality assurance process. In conclusion, HEMUA will improve the quality of the teaching system in the partner Universities by posing a model in which research, innovation and practical traineeships are key factors of the HE system.

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DOM and microbiome of olive tree cultivar Leccino are influenced by agricultural management in the Latium Region

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The olive tree is a plant that reacts differently to management practices and some experimental evidences reveal that plant age, cultivar and different soil managements often affect DOM (Dissolved Organic Matter) and microbial diversity.
DOM represents the most labile fraction of Soil Organic Matter and for the olive tree rhizosphere, can be considered an active C pool for carbon sequestration that can be affected by soil management, root exudates and vegetation changes. Understanding to what extent the root microbial community of olive trees is shaped by DOM input may contribute to a more complete knowledge of proper agronomic soil management in well definite biogeographic conditions. Therefore, the aim of this study was to assess the rhizosphere microbiome assembly and DOM in four "Leccino" orchards that were more than 30 years age, and that from long term, were affected by different agronomical management. Different olive orchards (cultivar Leccino) subjected to conventional management (CM), organic management (OM) and combined organic management with consociative cover crop (COMCC – Vulci site) were selected in Lazio Region (Central Italy). Metagenomic monitoring of rhizospheric soils, was performed by Next Generation Sequencing (NGS) and coupled by chemical characterization of Water-Extractable Organic Matter (WEOM) as chemical indicator of DOM. Data showed that the WEOM, in the Leccino rhizosphere managed with consociative cover crop (Vulci site), was higher respect to other treatments and in this trophic niche the microbiome, exhibited higher diversity. In Vulci site, the microbiome was dominated by bacteria from the phyla Actinobacteriota, Proteobacteria and Acidobacteriota. The Verrucomicrobiota was an ecologically relevant phylum that occurred in the rhizosphere of soils managed by conventional and organic practices (CM and OM). Regarding Actinobacteriota modulation we observed that the microbiome in the rhizosphere of olive grove with consociative cover crop (COMCC) resembled the structure of aged olive tree.

Soil prokaryotic response to animal manure digestate fertilization over time in a humid continental climate

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There is growing global interest in enhancing soil health through increased use of organic amendments, particularly animal waste–based digestates. This study aimed to assess the impact of different animal waste–based digestates on soil prokaryotic diversity and composition over three years in a cereal cropping system (spring wheat, triticale, and barley). The field experiment was performed in a randomized block design with five treatments (three replicates each): pig, chicken, and cow manure digestates; synthetic nitrogen fertilizer; and an unfertilized control. Prokaryotic soil communities were characterized by Illumina MiSeq sequencing. The dominant bacterial phyla across all treatments were Actinobacteria, Acidobacteria, and Proteobacteria, representing over 55% of the total prokaryotic community. Other notable phyla included Verrucomicrobia and Bacteroidetes, which formed part of the native soil microbiota. Additionally, digestate application did not significantly influence the prokaryotic diversity in the soil, with sampling time being a major factor in driving β-diversity. A positive correlation with soil pH was also observed for several taxonomic groups, indicating its importance in defining prokaryotic community composition. Our study found that digestate application did not significantly affect soil prokaryotic richness and diversity. Instead, crop type and seasonal/climatic variation were the primary drivers of prokaryotic community composition. Importantly, the animal manure digestate had a positive outcome by maintaining microbial stability.

A microbial-based strategy to mitigate kiwi vine decline syndrome and restore soil biological functionality

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Kiwi vine decline syndrome (KVDS) is an emerging multifactorial plant disease that negatively affects the productivity of *Actinidia* reducing also soil fertility. Given the possible role for an imbalance in kiwifruit root-associated microbiota in KVDS emergence, strategies that include the inoculation of beneficial microorganisms seem promising in alleviating this syndrome. In this work the microbial consortium consisting of the plant-growth promoting bacterial strains *Azotobacter chroococcum* 76A, *Priestia megaterium* EL5, *Methylobacterium populi* VP2, and *Kosakonia pseudosacchari* TL13 and the arbuscular mycorrhizal fungus *Funneliformis mosseae*, was used to evaluate their potential in improving Actinidia health, contributing to reduce the occurrence and the damages caused by KVDS. The inoculum was applied to one-year-old Actinidia-infected plants grown in greenhouse under waterlogging stress conditions. Un- inoculated and un-infected plants were used as controls. Plant fitness parameters recorded after exposing plants to abiotic conditions conducive for KVDS to measure both the incidence and severity of KVDS, indicated that healthy plants treated with beneficial microorganisms developed a more robust root system. Total genomic DNA extracted from endorhizosphere was used to investigate the impact of microbial consortium on specific bacterial groups involved in the key step of nitrogen cycle and phosphorous solubilization as well as to quantify specific fungal gene markers for functional symbiosis. qPCR revealed a significant ($P<0.05$) increase of the abundance of the phoD gene, involved in phosphorus solubilisation, in the soils treated with the beneficial consortium. These results suggest that the application of selected consortium appear promising in maintaining biological fertility of soils helping the plants also in stress conditions. This study provides a useful foundation for the implementation of biological strategies in the soil management and plant health.

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A microorganism-based biofertilizer for sustainable agriculture: scale-up studies for technological transfer

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The biostimulant market is rapidly expanding (CAGR > 10%), due to increasing demand for better yield and quality of agricultural crops. Agriculture is seeking for sustainable solutions that reduce over- and mineral fertilization. We developed a microorganism-based biostimulant (RhizoBact) within the project AlmaValue "RISORSA" with the aim at conceiving a new product for plant nutrition. The funding allowed the TRL (Technology Readiness Level) implementation (from TRL4 to TRL7), in order to achieve a prototype of our solution for the market players and/or spin-off creation. The one-year project looked at several horticultural crops (lettuce, basil, melon, black cabbage and fennel), strawberry and grapevine. In the present abstract we focused on lettuce since the study has been designed as a scale-up of a previous greenhouse experiment (Microbial Diversity 2023). In addition, two competitor products from the market were tested. Open field randomized experimental design was performed with four replicates (36 lettuce plants/replicate); four treatments were done by fertirrigation (at transplant and every week). Chlorophyll, leaves number and leaves surfaces were registered during the main phenological stages; at harvest, foliar and root fresh and dry weight, nutrient uptake and nitrates were analyzed. RhizoBact evidenced a significant increase in all phenological parameters compared to control and the competitor products. Lettuce productivity raised up to 32%, confirming the greenhouse results; moreover, the significant increase of root biomass showed the noteworthy role of the formula in promoting root development. Total nitrogen did not show significant differences among treatments, while a significant increase was found in several nutrients (K, P, Na, Ca, Fe, Mg, Mn, Cu) for all biostimulants. Interestingly, although not significant, a reduction of nitrates content in leaves was found. The formula is a successful application in the field of agricultural microbiology in the light of new product development for the biofertilizer market.

In vitro preliminary evaluation of potential biological control agents against *Plenodomus tracheiphilus*

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Mal secco disease of citrus is a highly destructive vascular disease caused by the necrotrophic fungus *Plenodomus tracheiphilus* (Petri) Gruyter, Aveskamp & Verkley. The pathogen, listed in EPPO's A2 list, is present in the Mediterranean basin severely affecting lemon trees, leading to significant losses. Currently, the only strategies available to reduce conidia inoculum in open field include the combined use of copper- based compounds and proper agronomic practices. However, due to the environmental impact of copper and the resulting restrictions imposed by the European Union, it is essential to explore eco-friendly solutions to manage the disease. This study aimed to isolate and evaluate microorganisms with potential antagonistic activity, through different in *vitro* methods, against *P. tracheiphilus*. Thirty-four isolates, including both bacteria and yeasts, were selected from various Mediterranean plant-based matrices, as well as from the microbial collection of CREA. The potential antagonistic activity of the isolates was evaluated using dual culture assays, both direct and indirect contact of isolates with *P. tracheiphilus*. Additionally, for each isolate, the antagonistic activity of cell-free supernatant (CFS) against the pathogen, was assessed by disk diffusion method, and by spreading the filtrates directly onto the plates. All assays were carried out in triplicate for each microbial isolate. The antagonistic effect was determined by calculating the percentage of mycelial growth inhibition compared to the control (fungus alone). On the whole, some of the tested microorganisms showed promising preliminary in *vitro* results encouraging further investigations aimed at validating their potential use as biological control agents to manage citrus mal secco disease.

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Effects of co-inoculation of *Pseudomonas sp.* strain NK2/7 and *Bacillus sp.* strain NK1/19 on plant growth, soil elements, and the relationships of soil microbiomes with soil physicochemical parameters

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To address declining agricultural productivity, the widespread reliance on chemical fertilizers has become a popular strategy globally to enhance crop yields. We therefore determined the potential of co-inoculation of the indole-3-acetic-acid- and ammonia-producing bacteria (*Pseudomonas* sp. strain NK2/7 and *Bacillus* sp. strain NK1/19) immobilized in agar to promote mustard greens growth and investigated the effects of both strains on plant and soil elements as well as soil bacterial community structure. The results show that the co-inoculation of both strains increased plant dry weight up to 62.02% and plant elements, including N (38.46%), P (28.37%), K (203.16%), Ca (10.89%), Mg (25.8%), and Na (70.3%). The co-inoculation also increased organic matter (OM) (by 429.45%) and increased soil elements including Fe (92.74%), Mn (126.88%), Cu (12.05%), and Zn (107.46%). High-throughput sequencing demonstrated that the establishment of both strains affected the soil bacterial community by reducing bacterial diversity and richness. Bacterial classes *Bacteroidia, alpha-Proteobacteria, Vicinamibacteria, Gemmatimonadetes, Verrucomicrobiae, Polyangia, Nitrososphaeria*, and *Blastocatellia* were significantly decreased, whereas the amounts of *gamma-Proteobacteria* and *Bacilli* were significantly increased. Soil parameters, including pH, OM, total Fe, Mn, Cu, and Zn, were the major factors influencing the soil bacterial community structure.

Diversity and composition of mycorrhizal simbionts and soil microbial communities are affected by floor management in a Mediterranean olive orchard

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Intensive and super-intensive olive cropping systems, combined with frequent tillage of orchard floors and the large use of chemical fertilizers and pesticides can heavily affect soil microbial communities. On the other hand, the use of cover crops and natural grass cover in olive orchards may provide several soil benefits and enhance the abundance, activity and diversity of soil microbiota. This work investigated the effects of cover crops, preceded by deep tillage, compared to undisturbed natural grass cover on the diversity and composition of arbuscular mycorrhizal fungi (AMF) and soil microbial communities in a Mediterranean olive orchard, over a two-year period. The natural grass cover treatment consisted of no-till management with diversified natural vegetation. For each treatment, three replicate composite soil samples were analysed using molecular approaches, Illumina MiSeq sequencing and PCR-denaturing gradient gel electrophoresis (PCR-DGGE). Molecular analyses identified 31 AMF taxa, predominantly from the Glomeraceae family (84%). Some AMF taxa demonstrated resilience to deep tillage, which was the main factor influencing AMF community composition during the first cropping season. The cover crop treatment significantly increased bacterial diversity, while fungal diversity remained stable. Our results indicate that cover crops preceded by tillage can be an effective and sustainable inter-row management strategy in olive orchards, supporting the short-term re-establishment of AMF communities and positively impacting bacterial diversity, thereby contributing to soil health and agroecosystem resilience.

Exploitation of fish wastewaters and agri-food waste and by products to produce microbial biomasses for bio based biostimulant

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The seafood industry generates 2 – 5 m3 wastewaters/ton of fish that currently are treated as waste. However, process waters contain valuable nutrients that have the potential to be recycled into biomass for different purposes such as microalgae for alternative protein extraction, or plant growth promoting microorganisms. One of the main limitations of the use of fish processing wastewaters as substrate is their composition variability that can affect the availability of nutrients provided by the bio-waste and can also show potential inhibitory effects for microbial biomasses growth. In the framework of the ReLEAF CBE project (GA n.101156998), different types of fish wastewaters provided by Ecopesce, SME involved in the project, were analyzed and adjusted in composition by fish and agri-food wastes to permit the growth of several Plant Growth Promoting Bacteria (PGPB) to be reused in chitosan-based formulations for Bio Based Biostimulants. The fish wastewaters were adjusted mainly in carbon source (0.5%, 1%, 2%), throughout the addition of molasses and peach by-product concentrate, and soluble protein content by the adding of fish waste hydrolysate. The modified wastewaters were used in microtiter model and inoculated at level of 6 log CFU/ml with different strains belonging to the Department of Agricultural and Food Sciences. The strain growth was monitored at 30 and 37 °C in Tecan. The growth raw data were modelled according to the Gompertz equation, modified by Zwietering, et al. (1990). The results obtained showed good potentialities of the microbial candidates which were able to reach optical density ranging between 1.8 and 2.2 OD. The used fish wastewaters were also analyzed before and after the growth of the strains for pH, total solids, total N, protreins, sugars, dry matter, BOD and COD and micronutrients in order to understand the percentage of biovalorization of the employed fish wastewaters.

Impact of herbicides and fungicides on soil bacteria: unveiling neutral to positive dynamics based on the soil and active substance

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Pesticides are essential for crop protection, but their adverse effects on beneficial soil microorganisms undermine the plants' natural defenses and disrupt crucial functions like nutrient cycling and soil fertility. Despite ongoing research, our understanding of the specific impacts of individual pesticides on microorganisms remains insufficient due to the many active ingredients on the market that frequently change. Here, we tested the immediate direct effects of three fungicides and five herbicides on the diversity, total abundance, and community composition of bacteria. We applied pesticides at concentrations recommended by manufacturers to three soils differing in organic matter content and pH, which had not been treated with pesticides for 10 years. To identify communities of bacteria, we used Illumina pair-end amplicon sequencing. To evaluate the total abundance of bacteria, we employed real-time PCR. We recorded positive, negative, and neutral effects of pesticides on the diversity and the total abundance of bacteria, depending on the active ingredient and soil used. As pH varied among the soils used, was influenced by pesticides, and in turn affected both total bacterial abundance and bacterial community composition, it can be concluded that the impact of pesticides on bacteria was at least partially indirect, mediated through changes in soil pH. Therefore, when selecting active ingredients, it is crucial to account for specific environmental conditions to minimize negative impacts on soil microbial communities and promote sustainable agriculture.

Isolation and characterization of rhizosphere fungi from olive roots for biostimulant production

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The In.Mi.Qu.Oil project, supported by the Sicilian Region, was developed to enhance the value of Monti Iblei PDO extra virgin olive oil, from the geographical area of Monti Iblei production zone, encompassing the provinces of Catania, Ragusa, and Siracusa. The main objective of the project was to support the regional olive-growing sector by developing and implementing an advanced and sustainable production model based on technological innovation. Through experimental research and implementation of protocols aimed at environmental sustainability, the testing of new methodologies to improve olive quality in the field, and the adoption of innovative processing technologies, the project aimed to make a significant contribution toward addressing critical issues within the olive oil value chain. The rhizosphere of *Olea europaea* represents a promising ecological niche for beneficial fungi with potential applications in sustainable agriculture. In this study, the isolation and morphological-molecular characterization of *Trichoderma* spp. strains, from the rhizosphere of olive trees cultivated in Southern Italy, were carried out. A total of six fungal isolates were obtained and screened based on their colony morphology, growth rate, and spore production. Molecular identification was performed using species- specific primers, confirming the presence of five strains of *T. harzianum* and one strain of *T. atroviride* among the isolates. The selected strains were further evaluated for their plant growth-promoting traits, including phosphate solubilization, siderophore production, and auxin-like activity (IAA production). Several isolates exhibited strong *in-vitro* biostimulant potential, supporting their application in the formulation of microbial-based products aimed at enhancing olive crop productivity and resilience. These findings highlight the role of olive-associated *Trichoderma* spp. as valuable bioresources for the development of eco-friendly biostimulants, contributing to the promotion of sustainable and regenerative agricultural practices.

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Shifts in rhizosphere microbial diversity following biostimulant application in vineyards

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Microbial communities are central elements in the integrity of vineyard ecological systems, contributing with nutrient availability, vine root elongation and resilience. However, the impact of biostimulant inputs in the structure and diversity of these communities has not been completely understood.

In this study, the ecological effect that a commercial biostimulant has over vineyards rhizobiomes was evaluated. Six vineyards sites were analysed, both before and after the treatment. The shift in communities and taxonomical diversity was determined using amplicon sequencing, considering bacterial and fungal populations. Physiological data from grapevine was also measured, including vigour, yield, and berry quality. When considered the effect of the treatment on all the sampled vineyards, only the beta diversity of fungal communities showed a significant variation. On the other hand, at site level was possible to observe changes in alpha diversity, reflecting a differential response likely due to microenvironmental variables and initial microbial communities composition and structure. despite the minimal changes at a broad scale, the presence of relevant bacterial taxas such as *Mortierella, Streptomyces* and *Bacillus* was possible to assess.

The impact of these ecological variations was also correlated with changes in the plant physiology. Furthermore, different trends in malic acid, nitrogen and sugar accumulation were founded between treated and non-treated plants. These suggest a functional relationship between rhizosphere microbial composition and plant metabolic status, although direct causation has not been yet established.

This suggest that biostimulant can play a relevant role in alter rhizobiome ecology through subtle but functional shifts in microbial diversity. Is important to evaluate and consider this finding in an ecological manner, recognizing microbial communities not only as soil health indicators but also as driven of crop yield and quality.

Exploring the diversity and use of native arbuscular mycorrhizal fungi for low-input agroecological weed menagement in European Living Labs

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Arbuscular mycorrhizal fungi, key plant soil beneficial symbionts, play a fundamental role in the transition towards low-input, safe and resilient agroecosystems. In particular, they can contribute to the reduction of agrochemicals, together with other Agroecological Weed Management (AWM) strategies. This work within the framework of the Horizon Europe project GOOD (AGrOecOlogy for weeDs) investigated the diversity of native AMF communities of 14 European Living Labs (LLs) from 7 different EU countries (the Netherlands, Serbia, Italy, Greece, Portugal, Spain, and Cyprus) to be used as seed inocula to promote the competitive ability of cover crops against weeds. Such native AMF communities were massively reproduced in pot cultures, given their status of obligate biotrophic symbionts. Moreover, a protocol was developed and tested on seeds of 11 plant species varying in shape, size and weight, to use seed inoculation as a means of delivering native AMF communities to cover crops. Morphotypes richness differed among EU LLs: the highest (17 AMF species) was found in Spanish LLs, while the lowest was detected in Italy and Cyprus (4-5 AMF species). Some species, such as *Entrophospora etunicata*, *Glomus badium*, *Pacispora sp*. and *Rhizoglomus spp*. were retrieved in 3-5 LLs, while spores belonging to Ambisporaceae, Archaeosporaceae, Acaulosporaceae and Scutellosporacerae were found at very low density in many LLs. Seed inoculation protocol was set up using different quantities of AMF inoculum, a commercial liquid adhesive material and water, to ensure uniform coverage of seeds. The protocol was then scaled up for on-farm application. Future studies will be carried out in order to assess mycorrhizal inoculum potential of LLs soils, the changes in the native AMF communities after cover crop inoculation and weed reduction.

Soil amendments in nematode suppression: a comparative study of native vs. bioprocessed food residues

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Ensuring crop health is essential to meet the growing demand for food and effectively managing root-knot nematodes (RKNs) can help maximize agricultural output. Indeed, plant diseases caused by RKNs belonging to the *Meloidogyne* genus, among the most harmful pests of horticultural crops, lead to severe productivity and economic losses. This study aimed at assessing the effectiveness of wasted bread, brewers' spent grain, and spent coffee grounds, native or bioprocessed, to suppress RKN *Meloidogyne incognita*, when used as soil amendments. Bioprocessing included the use of enzymatic treatments and fermentation with selected lactic acid bacteria or an incubation with compost tea. The matrices were incorporated to infested soil at doses corresponding to 3000 kg ha-1 organic carbon. Biomasses were characterized for their main physico-chemical and biochemical properties. Then plant growth and soil infestation were monitored to assess the biomasses potential as amendments. The different nature of the biomasses resulted in a supplementation in total nitrogen, phosphorous, organic acids and phenolic compounds strictly dependent on the food residue used and the bioprocessing employed. Although all the amendments significantly suppressed the multiplication of *M. incognita* and gall formation on tomato roots (up to 98 and 79%, respectively), the strongest suppressive effect was achieved by using bioprocessed brewers' spent grain, resulting in performances comparable to synthetic nematicides (79 eggs and juveniles g-1 roots). The nematocidal effect was ascribed to phenolic compounds and organic acids produced during lactic acid bacteria fermentation. Bioprocessed brewers' spent grain also resulted in the best growth effect on tomato plants.

Silverskin-carried microbial biostimulants boost maize growth and rhizosphere diversity

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Microbial-based biostimulants are a promising and sustainable approach to improve crop yield and preserve nutritional quality providing essential an alternative to synthetic fertilizers. The objective of this study was to develop an innovative and cost-effective microbial-based biostimulants and to assess their effect on the microbiota of the rhizosphere and endorhizosphere of maize plants. Five bacterial strains (*Priestia megaterium* EL5, *Azotobacter chroococcum* 76A, *Kosakonia pseudosacchari* TL13, *Methylobacterium populi* VP2, and *Streptomyces thermocarboxydus* C2_8A) were selected based on their plant growth- promoting activities. Three distinct bioformulates were developed: a powdered bioformulate (freeze-dried microbial cells); a liquid bioformulate (emulsion based on alginate and castor oil); and a granular bioformulate (obtained from solid-state fermentation on the coffee silverskin, a waste of the roasting process). Experimental tests were carried out on maize plants cultivated in greenhouse in accordance with the ministerial decree (DM 27/01/2014). After 21 days of plant growth, several biometric indices were assessed highlighting that the three biostimulants showed different levels of effectiveness. The microbial- coated silverskin appeared to be the most effective carrier for enhancing plant development since plants inoculated with silverskin showed a dry weight significantly higher than others. Chemical analyses of the rhizosphere showed that the plants treated with silverskin had significantly highest total nitrogen and assimilable phosphorus values. Moreover, the results about the microbiota dynamics in the maize rhizosphere and endorhizosphere revealed that the microbial-coated silverskin formulation had the most substantial impact on the microbial community in both environments. Solid-state fermentation on coffe silverskin effectively produces microbial biomass from food waste and develops biostimulants that enhance plant growth and soil biodiversity, offering an eco-friendly alternative to chemical fertilizers.

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Evaluation of the delivery of plant growth promoting bacteria to lettuce using biomaterials from by-products of the agri-food industry

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Agriculture contributes to environmental pollution and greenhouse gas emissions through the intensive use of plastic and agrochemicals. Also, the global food chain produces a great number of wastes. Plant Growth- Promoting Bacteria (PGPB) are well-known microorganisms associated with plants, representing a biotechnological alternative to reduce the use of chemical fertilizers. Nonetheless, challenges related to PGPB delivery and establishment in the soil still pose limits to the use of this promising technology. The aim of this work is to explore the delivery of selected PGPB using biomaterials that can be derived from by- products of the agri-food system, in a circular economy approach. Two previously characterized PGP strains, *Rhizobium sp*. GR12 and *Bacillus sp*. LR01 were subjected to additional screenings to test their tolerance to different abiotic stresses and their capacity to degrade biopolymers. Bacteria were embedded within liquid, pectin-based biomaterials, and their viability was assessed by re-isolating them after solidification and drying. *Bacillus sp*. LR01 was successfully re-isolated up to one month of biopolymer storage with an average concentration of 10^7 cfu/g of material. No survival was observed for *Rhizobium sp*. GR12, hence alginate beads were chosen as alternative delivery material. The ability of bacteria to promote plant growth was further assessed on *Lactuca sativa* in two independent experiments under greenhouse conditions. Lettuce plants were subjected to analysis aimed at verifying growth parameters and bacterial colonization. Strains LR01 and GR12 were successfully released from the biomaterials and effectively colonized the rhizosphere. Both strains delivered as liquid inoculant confirmed their ability to improve plant fresh weight and stem length, while their administration via biomaterials did not show any significant variation compared to the non-treated control. Our results indicate the importance to consider the formulation and/or employment methods of biomaterials to make them efficiently suitable for PGPB delivery in agriculture.

Rhizosphere microbiome transplants from Guinea grass for smallholders' application to chili and rice at field scale

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Smallholder farmers in the Global South are disproportionally affected by anthropogenic soil degradation and climate change, that affect agriculture productivity and threaten food security worldwide. Therefore, the need of more sustainable agricultural practices to improve crop resilience is on the rise. Rhizosphere microbiome transplant (RMT) is an emerging approach of plant microbiome engineering, for its potential to reduce the input of agrochemicals, hence preserving soil ecosystem and human health. However, its feasibility and ecological outcomes need deeper investigation, especially at field scale. This work, within an international cooperation project, aimed at evaluating the impact on crops yield and on soil microorganisms of a biofertilizer that could be self-produced by small hold farmers in Sri Lanka through RMT from Guinea grass (*Panicum maximum*), a widespread weed able to adapt to harsh conditions. Root wash (RW) and arbuscular mycorrhizae (AMF) were obtained from the root system of Panicum and separately supplied to chili pepper and two local varieties of rice, Suwadel and Kuruluthuda. Decreasing doses of chemical fertilizer were also applied, combined with the inocula or as separate controls. In chili and in rice var. Suwadel, RMT improved the crop productivity compared to the non-treated controls or to the plants supplemented only with the minimum dose of chemical fertilizer. RW and AMF applied alone or supplied with 50% of the optimal fertilizer dose resulted in yields comparable to 100% chemical fertilization, showing the potential to halve its input. 16S rRNA and ITS high-throughput sequencing showed an impact of RMT on bacterial and fungal communities at flowering stage, with the enrichment of Bacillaceae, Exiguobacteraceae, Micrococcaceae, Trichocomaceae and Aspergillaceae compared to non-treated plants. Our results indicate promising results of RMT in terms of crop yield improvement in field conditions, with structural changes in the rhizosphere microbiome possibly related to the recruitment of beneficial microorganisms.

A microorganism-based biofertilizer for sustainable agriculture: scale-up studies for technological transfer

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The biostimulant market is rapidly expanding (CAGR > 10%), due to increasing demand for better yield and quality of agricultural crops. Agriculture is seeking for sustainable solutions that reduce over- and mineral fertilization. We developed a microorganism-based biostimulant (RhizoBact) within the project AlmaValue "RISORSA" with the aim at conceiving a new product for plant nutrition. The funding allowed the TRL (Technology Readiness Level) implementation (from TRL4 to TRL7), in order to achieve a prototype of our solution for the market players and/or spin-off creation. The one-year project looked at several horticultural crops (lettuce, basil, melon, black cabbage and fennel), strawberry and grapevine. In the present abstract we focused on lettuce since the study has been designed as a scale-up of a previous greenhouse experiment (Microbial Diversity 2023). In addition, two competitor products from the market were tested. Open field randomized experimental design was performed with four replicates (36 lettuce plants/replicate); four treatments were done by fertirrigation (at transplant and every week). Chlorophyll, leaves number and leaves surfaces were registered during the main phenological stages; at harvest, foliar and root fresh and dry weight, nutrient uptake and nitrates were analyzed. RhizoBact evidenced a significant increase in all phenological parameters compared to control and the competitor products. Lettuce productivity raised up to 32%, confirming the greenhouse results; moreover, the significant increase of root biomass showed the noteworthy role of the formula in promoting root development. Total nitrogen did not show significant differences among treatments, while a significant increase was found in several nutrients (K, P, Na, Ca, Fe, Mg, Mn, Cu) for all biostimulants. Interestingly, although not significant, a reduction of nitrates content in leaves was found. The formula is a successful application in the field of agricultural microbiology in the light of new product development for the biofertilizer market.

Linking microbial extracellular electron transfer to arsenic dynamics in rice paddies

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Despite extensive research on engineered bioelectrochemical systems, little is known about the identity, metabolic pathways, distribution, and ecological functions of electroactive microorganisms in natural ecosystems. Microbial Extracellular Electron Transfer (MEET) is believed to play a pivotal role in the cycling of redox-sensitive elements such as arsenic, a major environmental and food safety concern in flooded rice paddies. This study aims to establish a structured methodology for investigating the contribution of MEET to biogeochemical cycling in wetlands, using arsenic transformation in rice paddies as model system. To assess the impact of increased soil conductivity on the proliferation and activity of electroactive microorganisms, a controlled microcosm experiment was set up with flooded arsenic-contaminated rice paddy soil amended with electroactive biochar. A closed electrical circuit was established connecting a buried anode connected to a floating cathode, allowing real-time monitoring of extracellular electron flow. Anodic microbial communities will be characterized by metatrascriptomic profiling and Real Time qPCR. Preliminary findings showed that biochar-amended soils exhibited significantly lower chemical oxygen demand and bacterial 16S rRNA gene copies compared to unamended controls (250 vs 40 mg L-1 and 108 vs 103 gene copies g-1 soil). Nonetheless, microbial communities enriched in biochar-amended microcosms demonstrated significantly higher current transfer efficiency (1×10-4 vs 3×10-5 A gene copies-1), indicating enhanced extracellular electron transfer relative to controls. These results suggest that increasing soil conductivity promotes the presence and activity of electroactive microorganisms, supporting further investigation into the role of MEET in arsenic transformations within rice paddy environments.

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Colonization of biological control agents (BCAs) for the management of mal secco disease through culture dependent and - independent techniques

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In plant production, biocontrol agents (BCAs) are increasingly used as alternatives to synthetic fungicides to manage plant pathogens. Specifically, *Bacillus* spp. and *Trichoderma* spp. are widely investigated against *Plenodomus tracheiphilus*, the causal agent of mal secco disease. This study aimed to evaluate the colonization capacity of selected commercial *Bacillus* and *Trichoderma*-based products in different plant tissues. Experiments were conducted on Volkamer lemon seedlings, with three biological replicates (15 seedlings each), foliar-treated with BCAs and sampled at 7, 14, and 21 days for leaves and stems, respectively, using both culture-dependent (plate count) and culture-independent (qPCR) methods. In addition, rhizosphere was sampled from *Trichoderma* spp. root-treated seedlings by the same approaches. For the *Bacillus*-based product, culture-based methods showed colonization levels of 2.43 ± 0.21 Log CFU g^{-1} in leaves and 4.64 ± 0.10 Log CFU g^{-1} in stems at 21 days, while qPCR indicated higher values: 6.46 ± 0.13 and 7.72 ± 0.56 Log CFU g^{-1}, respectively. For *Trichoderma*, plate counts revealed low colonization in leaves (<1% isolation) but high levels in stems (up to 56%), supported by qPCR results of 5.42 ± 0.11 and 5.73 ± 0.01 Log conidia g^{-1}, respectively. In rhizosphere samples, *Trichoderma* showed persistence with values of 3.01 ± 0.01 (plate count) and 8.60 ± 0.35 (qPCR) Log conidia g^{-1} at 21 days. These findings confirm BCAs' ability to colonize plant tissues, especially the vascular system, which supports their persistence over time.

The research activity was carried out within Agritech National Research Center (European Union Next-Generation EU, PIANO NAZIONALE DI RIPRESA E RESILIENZA, PNRR – MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.4—D.D. 1032 17/06/2022, CN00000022, CUP: E63C22000960006) Spoke 2 (Task 2.2.4: 'Biopesticide and biostimulats').

Regenerative recycling of agro food waste for enhancing the Mediterranean food system's circularity

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Agriculture-based industries produce a vast amount of byproducts every year. If these residues are released into the environment without proper disposal, they may cause environmental pollution and harm human and animal health. Most agro-industrial wastes are untreated, underutilised, and generally disposed of by burning, dumping or unplanned landfilling, creating different ecological problems by increasing the number of greenhouse gases. Several Mediterranean agri-food side streams have a unique structure and nutritional composition with encrypted potential functional and bioactive properties (e.i, antioxidant, antifungal, gut- modulatory effects) which can be used to develop innovative and nutritious food products. The availability of valuable nutrients in these underexploited raw materials is often impaired by the presence of antinutrients (phytic acids, protease inhibitors, tannins and fibres) and the high compartmental nature of the plant cell structure. Those characteristics diminish the nutritional and biological value of agri-food by-products. Biotransformation strategies through fermentation represent a cost-effective strategy to overcome such challenges and strengthen the food circularity dimension. This work aimed to study the optimal conditions for setting a solid-state fermentation (SSF) of different and complementary agri-food by-products in order to optimise the degradation of their lignocellulolytic component.

For this scope, the edible mushroom *Pleurotus ostreatus* was used as a fermentative white rot fungus. Various substrates were first evaluated for the formulation of the fungal spawn (pre-culture). Subsequently, environmental parameters such as aeration, humidity, and temperature, as well as the best combination of the agro-food waste, were monitored and adjusted to ensure optimal conditions for fungal growth and colonisation.

At the end, the rate of lignocellulosic degradation was assessed through enzymatic assays and near-infrared (NIR) analysis.

Session 4

Microbial ecosystems, Environmental sustainability

Novel insights into the microbial diversity and ecology of global wastewater treatment and resource recovery systems

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Complex uncultured microbial communities carry out wastewater treatment, which often includes water reuse, resource recovery, and energy production. Despite a lot of research over the years, only lately, we have got a real breakthrough in the understanding of identity, function and ecology of the process-critical microbes. I will present new results from more than 750 treatment plants and 240 anaerobic digesters across the world, the retrieval and annotation of more than 20,000 high-quality metagenome assembled genomes, and development of comprehensive sets of FISH probes for the visualization and in situ studies of ecophysiology. Examples of insights into novel species, physiologies, global distribution, seasonal variation, and main drivers for community assembly, such as microbial immigration via the sewer system, will be presented. It all provides a shared resource in microbial ecology via the MiDAS webpage, providing new opportunities for cross-study comparisons and ecological studies at high taxonomic resolution, essential for future informed management of wastewater treatment systems.

Isolation and molecular characterization of metabolites produced by an Antarctic fungal strain

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Antarctica's microbial diversity is largely unexplored, but its microorganisms, adapted to extreme conditions, hold great potential for biotechnological applications. These extremophiles have evolved unique strategies, including changes in gene regulation and metabolic pathways, making them a valuable source of bioactive molecules. Extremolytes and secondary metabolites produced by Antarctic fungi are noteworthy for their anticancer, neuroprotective, and antioxidant properties.

This work deals with fungal strains isolated in Antarctica, which have demonstrated potential for therapeutic applications. These microorganisms, that were initially studied as a source of phenols and other antioxidant molecules, manifested their ability to inhibit the formation of amyloid fibrils of α-synuclein involved in the development of Parkinson's disease. Therefore, we decided to focus on the isolation and characterization of molecules responsible for this activity. By using chromatography techniques coupled with mass spectrometry, some products were extracted and tested *in vitro* and in cell systems in comparison to other molecules for of in-depth structure-activity relationship.

Due to the critical demand for alternative treatments of neurodegenerative disease, natural products could offer essential starting points for the generation of novel pharmaceuticals. In fact, these molecules, for their inherent bioactivity and structural diversity, could be used as natural scaffolds as promising candidates for the development of innovative treatments for Parkinson's disease.

Impact of mowing frequency on soil microbial diversity of urban roadside lawns

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Roadside lawns are a dominant form of urban greenery, yet their ecological potential is often compromised by overly intensive maintenance, particularly frequent mowing. Our project investigates the microbial aspect of this issue, identifying a previously overlooked research gap: how mowing frequency influences the structure of microbial communities in roadside lawn soils.

The study was conducted in Katowice and Cracow, two major cities in one of the most polluted regions in Europe. A large-scale field experiment was carried out across four sites subjected to three mowing regimes: high (6 cuts/season), medium (3 cuts/season), and low (1 cut/season). Soil and vegetation samples were collected along a gradient of traffic-related pollution (proximal vs. distal to roads). Soil bacterial and fungal communities were characterized using short-reads sequencing of the 16S rRNA gene and the ITS region. The microbial communities of each of the sites studied were significantly different. Obtained results indicate that within each site, the soil contamination - particularly from road-derived pollutants - is the primary driver of alpha and beta diversity in soil microbial communities. Mowing frequency had a smaller and more localized effect on soil microorganisms. Nonetheless, microbial community shifts linked to mowing regimes were observed, especially in fungal community, suggesting that mowing frequency affects the structure of soil microbial communities, but not to the extent assumed before the study began. The results underline the impact of prolonged contamination, the effects of which are reflected in microbial diversity despite different lawn treatments, which took place within the span of just one year. This study was a part of ToBeLawn project that bridges fundamental and applied research by translating the biodiversity of plants and microbes into actionable urban policy. The implementation of biodiversity-friendly mowing practices, if applied consistently, is expected to increase ecosystem resilience to urban stressors.

Fungi and plastisphere: can this biodiversity hide biotechnological powerful biocatalysts?

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Despite the current efforts to limit the impact of plastic on the environment, we cannot oversee the actual risk the entire ecosystem is exposed to. The mycobiota associated to the plastisphere is poorly-studied, with very few information available about its degrading skills. Our research purpose is to investigate extreme polluted environments, including plastic-polluted landfill soil and organic waste treatment plants, as well as marine water and sediments. The microbial community was studied through culturomic and metagenomic approaches, focusing the attention of both traditional polymers and bioplastic. The isolated fungal community differed a lot, with a strong role of the site, the plastic material and environmental parameters as the salinity. Hundred strains were isolated, mostly associated with the *Ascomycota phylum*. Despite the filamentous fungi were very abundant, yeasts were not neglectable (approx. 10%). Studies confirmed that environmental parameters differently affect the microbial community. Populating the plastisphere cannot be considered as an axiom of being capable of transforming plastic.

Studies were focused on the evaluation of this phenomenon: growth screenings were followed by liquid culture to quantitatively measure the polymer transformation yields. Many fungal strains (i.e. *Alternaria, Aspergillus, Cladosporium, Fusarium, Penicillium, Purpureocillium*) showed the capability to degrade plastic polymers with the consequent production of monomers. Fungi were also tested against different polymers and in peculiar environments, including sea water, agricultural soils and organic treatment plants. In a model marine environment, selected fungi did transform bioplastics, even though halving the yields obtained in fresh water. Moreover, when used as pre-treatment of plastic for further anaerobic digestion, they helped to strengthen and fasten bioplastics degradation. Fungal biodiversity has indeed proven to be involved in the transformation of plastic materials, resisting the harsh working conditions, but the involved metabolic pathways has still to be discovered.

Rebuilding belowground biodiversity: AMF and cover plants drive VOC regulation in urban soil restoration

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Urbanization, exacerbated by climate change, intensifies soil degradation, leading to losses in microbial diversity and disruption of essential ecosystem functions. To counteract these effects, ecological de-sealing and bioremediation strategies are essential. In this study, we conducted a mesocosm experiment using de- sealed soil from an urban area as a substrate. We tested the combined effects of inoculation with arbuscular mycorrhizal fungi (AMF) and two cover plant species—*Cynara cardunculus* L. and *Trifolium repens* L.—on volatile organic compound (VOC) emissions and soil microbiome dynamics.

Results revealed that VOC emissions were dominated by anthropogenic compounds, particularly arenes and furans. Notably, *C. cardunculus*, both with and without inoculation with AMF, was correlated with reduced levels of these compounds, indicating potential for phytoremediation and soil health restoration. AMF - *C. cardunculus* association significantly increased soil bacterial alpha diversity and shifted microbial ratios (e.g., Proteobacteria/Acidobacteria, Cyanobacteria/Chloroflexi, and Fungi/Bacteria), reflecting improved microbial balance and functional potential. Taxonomic analyses identified Chloroflexales and genera such as *Ralstonia*, *Delftia*, and *Ramlibacter* as key contributors to VOC degradation, underlining their adaptability to polluted urban substrates. Overall, our findings highlighted the synergistic role of AMF and selected cover plants in enhancing microbial diversity and functional recovery in degraded urban soils. This approach offers a promising strategy for post-de-sealing ecological restoration in urban environments.

Spontaneous bio-recycling: recovering bioactive molecules through endogenous microbial maceration of hemp residues

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Biomass residues represent a major issue for industries. On the other hand, residues enclosed major classes of bioactive compounds that could be extracted and used across various fields. This study aimed to elucidate the role of the endogenous microbial community in the lignocellulosic degradation of hemp residues for biorefineries or other industrial processes, simultaneously characterizing the composition of three extracts recovered at different stages of maceration. The process was examined from different perspectives: plant tissue degradation and microbial dynamics were monitored using histological, cultural-dependent, and independent analysis. Extracts were characterized through FTIR, NMR, and SDS-PAGE analyses, also evaluating their potential as biostimulants for microbial growth. Results revealed that the composition of the endogenous prokaryotic community remained stable during the maceration period, while fluctuations in various fungal genera were observed. The taxonomical composition of hemp residues at different stages may account for the increased accumulation of amide-containing compounds, sugars, and other metabolites detected in long-term bioconversion. Finally, the extracts recovered from the microbial degradation of hemp residues were able to support the growth of the yeast *Cryptococcus phenolicus* and the bacterium *Peribacillus simplex* as the sole source of nitrogen, paving the way for their potential use as biostimulants.

Development of a biotechnological method for enhanced oil recovery from exhausted oilfields on the Absheron Peninsula

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With many onshore oilfields in Azerbaijan nearing exhaustion after over a century of extraction, there is an urgent need for more effective and sustainable recovery methods. This study focused on developing a microbial enhanced oil recovery (MEOR) technique adapted for the high-temperature, water-saturated conditions typical of reservoirs on the Absheron Peninsula.

Thermotolerant microbial strains were isolated from oil-contaminated soils and formation waters. Eleven strains were selected based on their ability to grow at high temperatures and utilize hydrocarbons. Among them were *Bacillus* sp. 21, *Nocardia* sp. 14, *Pseudomonas* sp. 24, *Rhodococcus* sp. 7, and *Arthrobacter* sp. 9. These strains demonstrated the capacity to reduce surface tension from 55 to 42 dyn/cm, emulsify oil (up to 52%), and produce CO_2 at rates up to 95 mg/mg biomass per hour. They also generated biosurfactants, acids, and biopolymers—agents known to enhance oil displacement.

Locally available agro-industrial wastes, including whey, olive oil rinse water, and yeast production wastes, were tested as nutrient sources. The combination of lactoserum and olive rinse water (8:2 ratio) supported strong microbial growth and metabolite production. Laboratory model experiments confirmed that the selected strains and nutrient mixtures increased oil recovery by 12–14% compared to controls.

The proposed method not only offers a low-cost and environmentally sound alternative to traditional recovery techniques but also provides a productive use for organic industrial wastes. This work lays the groundwork for the wider application of MEOR in Azerbaijan's aging oilfields and contributes to the development of a circular bioeconomy in the region.

Study of mycobiota in honeybee ecosystem through fermentation monitoring

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Honeybees (*Apis mellifera*) are essential for biodiversity and food production. Beyond honey, one of the most valuable products used by humans is bee pollen. However, due to the outer wall of pollen grains, most of their bioactive compounds are not available for human consumers. Honeybees have developed a strategy to pass this problem thanks the help of microorganisms; inside the hive, over the course of one or two weeks, bee pollen evolved through a fermentation on bee bread which is more digestive and stable during the time.
The aim of the study was to fill the knowledge gap regarding the yeasts associated with honeybee system investigating more deeply the overall microbial consortium involved in the transformation of bee pollen into bee bread, and to identify a potential core mycobiota within the honeybee environment. For this, a preliminary ecological map was established sampling flowers, bee gut and hive products. Subsequently, a solid-state fermentation, using pollen and honey harvested from different hives coming from different sites of Marche region were carried out.
After ecological monitoring, a total of 27 yeasts genera and 51 species were identified. Basidiomycetes were predominant in flowers, while *Aureobasidium sp.*, *Filobasidium sp.*, *Meyerozyma sp.*, and *Metschnikowia sp.*, were found in all environments. Fermenting yeasts, such as *Debaryomyces sp.*, *Saccharomyces sp.*, *Starmerella sp.*, *Pichia sp.*, and *Lachancea sp.*, were mainly found in the gut, whereas most species identified in bee products were absent in gut mycobiota. Moreover, the yeast species *Meyerozyma guilliermondii, Debaryomyces hansenii, Hanseniaspora uvarum, Hanseniaspora guilliermondii,* and *Starmerella roseus* were detected in the bee gut during both summer and winter sampling, suggesting their role as a stable eukaryotic component of the core gut microbiota. Solid state fermentation revealed a complex interaction among bacteria and yeast populations that cooperated in a natural succession based on symbiotic relationships.

Harnessing novel indigenous bacterial potential for biodegradation of end-of-life tires using immobilized bioreactor

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According to Blue Wave Consulting (2022), the global tires market was valued at USD 276.36 billion in 2022 and is projected to reach USD 712.56 billion by 2030. This rapid expansion contributes to the increasing accumulation of end-of-life (EOL) tires, which present serious environmental challenges due to their complex, durable, and non-biodegradable composition. Among various recycling approaches, microbial degradation offers an eco-friendly alternative. Certain microorganisms can biotransform the synthetic polymers and additives in tires, leading to the assimilation and mineralization of resulting degradation products. However, the vulcanized rubber resists microbial attack due to its hydrophobic and highly cross- linked structure formed during vulcanization process. This study investigates the biodegradation potential of indigenous bacterial strains (single and mixed cultures) isolated from soil collected at tire dumping site in Goa, India. The soil inoculum were cultured in sulfur-free mineral salt medium supplemented with tire crumbs as carbon source. The bacteria were isolated using selective enrichment process and taxonomically identified using 16S rRNA sequencing. Sulfate ions released during biodegradation were quantified using ion exchange chromatography, indicating devulcanization. SEM-EDS analysis revealed surface roughness and pores and changes in elemental composition (C, O, S, Si, Zn), while ATR-FTIR confirmed modification of functional group, including C=C stretching (600–800 cm^{-1}), S=O stretching (1000–1150 cm^{-1}), and O–H stretching (3400–3700 cm^{-1}). TGA confirmed degradation across additive (100–300 °C), polymer (300–500 °C), and ash (500–600 °C) phases. CHNS analysis revealed significant reductions in carbon and sulfur content, indicating microbial utilization. Degraded oligo-isoprenes derivatized with 2,4-DNPH were detected using HPLC, and additives were identified using GC-MS. The process initially optimized at bench scale (250 mL flask,) was successfully scaled up in a 5L fermenter (2.5L volume), yielding higher degradation rates, demonstrating field deployment. Overall, the findings highlight microbial biodegradation as a promising strategy for sustainable tire waste management and environmental remediation.

Fungal diversity, a nature-based solution for rare elements recovery: investigation on interactions between fungal strains from culture collections and elements of strategic interest

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Fungal biodiversity represents a promising bioresource for the sustainable recovery of critical elements essential to high-tech applications. Indeed, due to their ability to tolerate, bioaccumulate, and transform toxic elements, fungi are valuable agents in eco-friendly processes for electronic waste recovery. "FunMetals" project explores interactions between selected fungal strains and strategic elements, supporting the circular economy and the ecological transition through low-impact, biologically-driven recovery systems.

This study focused on 24 fungal strains preserved in the culture collections of the Fungal Biodiversity Laboratory at Sapienza University of Rome and the National Research Council. Tolerance screenings were performed on solid medium in the presence of Gallium, Germanium, Indium and Yttrium oxides individually and evaluated by tolerance indexes based on mycelial weight and colony area. Medium's pH variation and secondary biominerals precipitation were also assessed. To evaluate element bioaccumulation and assimilation, the biomass of fungi was analyzed using Energy-dispersive X-ray fluorescence (XRF). The fungi were then tested for siderophores and laccase production. Moreover, a modified microculture system that allows for a multimodal imaging approach and SEM-EDS analysis was employed to investigate fungal assimilation and transport of elements through hyphae, as well as biomineral precipitation. The Phenotype MicroArray™ was then used to gather information on the effects of single elements on fungal metabolism and the metabolic performance of selected strains with and without the elements.

Information on the effects of different nutrients on fungal tolerance to metals and the effects of metals on the assimilation and use of carbon sources by fungi was obtained. The results highlighted that each strain had specific mechanisms of interaction with the elements and different degrees of tolerance, bioaccumulation, and biomineralization.

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Biological disinfection of domestic wastewater using moving bed biofilm reactor with saturated sand filter and its metagenomic analysis

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Traditional wastewater disinfection methods, such as ultraviolet (UV) irradiation and chemical treatments, often pose environmental risks and can be cost-prohibitive for widespread application. To address these challenges, this study employs an advanced, integrated treatment system implementing a Moving Bed Biofilm Reactor (MBBR) coupled with a Saturated Sand Filter (SSF) with a particular focus on the biofilm formed on the biocarrier and *schmutzdecke* layer formed on the surface of the SSF to achieve pathogen removal in domestic wastewater. The MBBR served as a secondary treatment step, while the SSF provided tertiary polishing. The combined MBBR-SSF system achieved significant reductions in Total Coliform (TC), decreasing concentrations from 4.3 ± 0.2 to 1.18 ± 0.97 log CFU at a 15 L/d flow rate (99.68% reduction) and from 4.50 ± 0.33 to 1.37 ± 0.98 log CFU at 30 L/d (99.79% reduction). In addition, the system demonstrated high removal efficiencies for key water quality parameters: at 15 L/d, Chemical Oxygen Demand (COD) and Biological Oxygen Demand (BOD) were reduced by 95.07% and 94.73%, respectively, while Total Organic Carbon (TOC), Total Carbon, and Total Nitrogen (TN) removal reached 79.78%, 80.32%, and 76.61%. At 30 L/d, removal efficiencies remained robust, with 87.86% for COD, 85.64% for BOD, 73.01% for TOC, 74.64% for Total Carbon, and 72.76% for TN. Microbial community analysis revealed that *Proteobacteria* dominated both treatment stages (55% in MBBR, 85% in SSF), followed by *Bacteroidetes*, *Planctomycetota*, and other minor groups. Collectively, these findings substantiate the MBBR-SSF system as a robust, scalable, and environmentally benign alternative to conventional disinfection. The synergistic integration of biological and physical processes not only ensures comprehensive pathogen inactivation and nutrient removal but also aligns with global imperatives for sustainable wastewater management.

Evaluation of gas emissions and volatilome in agricultural soil microcosms under induced denitrification: integrated analysis and microbial response

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Nitrogen is a critical nutrient for the productivity of agricultural ecosystems. However, most of the available nitrogen can exit agricultural systems leading to economic loss and environmental damage. For a better N management, the N-emissions need to be accurately measured, to develop indicators that can reflect the complexities of N cycling and linking them to biological processes that occur in agricultural soils. Therefore, the goal of this study was to measure soil volatilome in artificially induced denitrification conditions as useful information for the improvement of field sensors as metal oxide (MOX) sensors. Experiments were carried out using lab-based soil microcosms to measure nitrogen-cycle related emissions in two conditions: control soil and a boosted soil for denitrification in anaerobic conditions. Gas emissions were measured by Gasmet-FTIR. The soil volatilome in soil microcosms was evaluated with a MOX sensors device and simultaneously analyzed by GC-MS analysis at different time points. Lastly, to gain insights into the N-cycling process and N-emissions, abundances of the microbial N-cycling functional genes were analyzed by Quantitative-PCR (qPCR). The applied treatment successfully boosted the denitrification, hinting that the experimental setup was a reliable proxy to mimic denitrification process in agricultural soils. Specifically, this treatment stimulated the NOx emissions within the first 24 hours, achieving the highest N_2O emission rate at 24 hours after treatment. Overall, LDA analysis showed a clear separation over time between volatile organic compound (VOC) profiles with a significantly higher concentrations of alkanes, aldehydes and alcohols than the control in the boosting treatment. Genes abundances, the N_2O production and reduction potentials confirmed that denitrification process was induced earlier in boosted samples compared control samples.

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Harnessing desert cyanobacteria to support sustainable human space exploration

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Long-duration crewed missions on the Moon and Mars demand sustainable technologies that minimize reliance on Earth-based resources; hence the development of Life Support Systems (LSS) is mandatory. On Earth, cyanobacteria perform oxygenic photosynthesis and carbon dioxide fixation, processes that play a key role in Biological Life Support Systems (BLSS) off the planet. An important aspect is the utilization of local materials, the so-called in-situ resource utilization (ISRU). A link between local resources and BLSS components, otherwise unable to live 'off-the-land' is provided by lithotrophic cyanobacteria. Among them members of the *Chroococcidiopsis* genus isolated from extreme deserts are gaining increasing interest as key enablers to transform minerals, carbon dioxide and urine (from crew waste) into biomass to be used as bacterial feedstock for downstream production of consumables, or as plant biostimulant /fertilizer. Taking advantage of a multidisciplinary approach, we investigated the possibility to support bacterial growth by using the biomass produced by a desiccation, radiation tolerant cyanobacterium, namely *Chroococcidiopsis* sp. CCMEE 029, cultivated using simulants of Lunar and Martian soil supplemented with urea as a nitrogen source. At the same time, we investigated the potential role of *Chroococcidiopsis* strains capable of far-red light-acclimation to overcome regolith shading and support an increased biomass production. Notably, the cultivation of microorganisms using Martian regolith will be harmed by the presence of perchlorates, that being strong chaotropic agents, destabilize macromolecules and trigger oxidative stress. The capability of *Chroococcidiopsis* sp. CCMEE 029 to cope with perchlorate concentrations exceeding those naturally occurring in dry environments was evaluated and correlated to pathways associated with desiccation tolerance. Finally, the proteomic investigation of *Chroococcidiopsis* sp. CCMEE 029 after cultivation in the presence of increasing perchlorate concentrations revealed that it reprograms its metabolism to cope with photosynthesis impairment by upregulating the ribulose monophosphate pathway and a vast repertoire of ROS-coping mechanisms along with the synthesis of proteins related to polyhydroxybutyrate production. By providing novel insights into the cultivation of desert cyanobacteria with Lunar and Martian regolith simulants, the obtained results advance our understanding of microbial adaptation to non-Earth environments and lay the groundwork for optimizing microbial biotechnologies in space exploration.

The keratinase-collagenase complex from an indigenous *Bacillus paralicheniformis*: a solution for valorizing hard-to-degrade protein wastes in Kazakhstan's agro-industry (wool, feather, and slaughterhouse waste)

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The livestock industry is instrumental in the provision of animal protein; however, it also produces a significant amount of waste, with up to 45% of an animal's live weight being unsuitable for food consumption. In Kazakhstan, over 800,000 tons of these by-products are produced annually, including wool, feathers, hooves, antlers, and hides. These materials are abundant in structurally resilient proteins such as collagen and keratin. Poor solubility and mechanical robustness restrict their potential for reuse.

Enzymatic hydrolysis provides an economically and environmentally sustainable alternative for the conversion of these materials into value-added products. We describe the characterization of a *Bacillus paralicheniformis* strain, isolated from soil in southern Kazakhstan, that generates an effective protease complex with extensive substrate specificity. Using the optimal conditions (60°C, pH 9.0, 0.1 M Tris-HCl buffer), the crude enzyme extract showed high caseinolytic activity (249.1 U/mL) and keratinolytic activity (116.2 U/mL). It was proven that the enzymes could break down collagen by using collagen as a target in zymography. The presence of each of the three types of proteolytic activity allowed for effective hydrolysis of protein-rich slaughterhouse by-products, such as feathers (100% degradation), hide (97.2%), wool (34.5%), hooves (29.6%), and horns (3.6%) after 168 hours of incubation. The secretion of protease was also effectively induced in culture by wool and feather substrates.

The potential of this indigenous *Bacillus* strain and its enzyme complex to valorize poorly degradable agro- industrial residues through sustainable biotechnological methods is shown by these findings.

Tolerance of the adapted strain *Basfia succiniciproducens* 4D to high concentrations of *Arundo donax* hydrolysate for the production of organic acids

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The bio-based production of succinic acid from lignocellulosic biomass is one of the most promising alternatives to reduce dependence on fossil resources. Green biotechnologies require microbial strains capable of efficiently fermenting lignocellulosic hydrolysates, while tolerating inhibitory compounds released during biomass pretreatment. Among the most suitable feedstocks, *Arundo donax*—a high-yield perennial plant cultivable on marginal land—offers a low-cost and abundant carbon source for bio-based processes. Was used the adapted strain *Basfia (B.) succiniciproducens* 4D, derivative from the wild-type strain *B. succiniciproducens* BPP8. This is tolerant to both pretreatment-derived inhibitors and fermentation- derived organic acids, and has shown promising succinic acid productivity. Fermentations were carried out in MiniBio bioreactors using A. donax hydrolysate as the sole carbon source at concentrations of 5% and 30% (v/v), under specific controlled conditions (48 h at 37°C, 150 rpm, CO_2 sparging at 20 mL/min). The best yield (0.68 g/g) was observed after 48 h with 30% hydrolysate. To further enhance production, strain 4D was tested with increasing hydrolysate concentrations (50% and 90%) up to 72 h, to assess its tolerance and performance under higher inhibitory pressure. 4D cultivated with 50% hydrolysate reached an optical density (OD) of 0.282 after 48 hours, and a maximum yield succinic acid of 0.128 g/g and of acetic acid of 0.229 g/g. At 90%, OD reached 0.398 at 72 h, with succinic acid yield of 0.083 g/g and acetic acid of
0.294 g/g. These findings confirm the suitability of *B. succiniciproducens* 4D for high-efficiency industrial bioprocesses based on lignocellulosic feedstocks, contributing to the development of competitive and sustainable bio-based value chains.

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Novel isolation campaign to explore microbial biodiversity associated to a Pied de Cuve for Amarone wine

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Pied de Cuve (PdC) is a winemaking technique where a small amount of fermenting must is used to start the main fermentation and spontaneous *PdC* fermentation, driven by resident microbiota, is considered to positively express the *"terroir"* concept. The study explores the microbial biodiversity of spontaneously fermented *PdC* for "Amarone della Valpolicella" wine through culture-dependent methods, to determine presence, dominance and persistence of winemaking-related microbiota. Nine samples were collected during *PdC* fermentation and after inoculum in total mass. Non-*Saccharomyces* yeasts were isolated from *PdC* after grape crushing and before inoculation. *Saccharomyces* and bacteria were sampled during the *PdC* fermentation at 12,8% alcohol (v/v) and after inoculation. Cell counts were performed on WL and MRS agar. The cell population remained around 5.5 log CFU/mL until the beginning of alcoholic fermentation in PdC, reaching a peak of 8 log CFU/mL at 1.89%. Colonies from countable plates were selected based on morphology and differentiated into *Saccharomyces* or non-*Saccharomyces* yeasts using lysine agar, resulting in 118 isolates: 26 putative non-*Saccharomyces* yeasts, 89 putative *Saccharomyces* yeasts, and 3 bacteria. Dominance and persistence of different *Saccharomyces* strains during fermentation were evaluated with Interdelta-targeted PCRs, while non-*Saccharomyces* isolates were dereplicated and identified through sequencing of the D1/D2 domains of 26S rRNA gene. This study highlighted, for the first time, the biodiversity associated with spontaneous *PdC* fermentation in Amarone wine and identified the dominant *Saccharomyces* strains, which will be further characterized for enological traits.

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Exploring the power of impedometric analysis: a cutting-edge approach to evaluate antimicrobial activity of natural extracts and essential oils

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The increasing demand for natural and sustainable solutions in food preservation has driven renewed interest in essential oils (EOs) and plant extracts (PEs) for their antimicrobial and antioxidant properties. Within the framework of the project "Bioactive compounds to Extend food Shelf-life through Innovative Technologies (BEST)", this study aims to identify valuable bioactive compounds from underutilized or waste materials, aligning with the principles of Circular Economy and sustainability to combat food waste. The antimicrobial and antioxidant potential of lemon, lavender, and oregano EOs, along with tomato and artichoke extracts, was evaluated against key foodborne and spoilage microorganisms. An impedometric approach was applied to assess their activity against *Listeria innocua, Escherichia coli, Pseudomonas fluorescens, Zygosaccharomyces bailii*, and *Kluyveromyces marxianus*. While tomato and artichoke extracts exhibited no antimicrobial activity (MIC > 66.67 μL/mL), all tested EOs were effective to varying extents. Oregano EO stood out for its strong bacteriostatic and bactericidal effects across all tested strains, even at low concentrations. Lavender and lemon EOs showed moderate to limited efficacy. Notably, impedometric analysis proved highly sensitive for evaluating antifungal activity, highlighting lavender EO as the most effective against yeasts (MIC/MFC = 0.75 μL/mL). Antioxidant activity, measured via DPPH and Oxitest assays, was also highest in oregano EO, suggesting a possible link between antioxidant and antimicrobial effects. These results not only support the use of impedance-based techniques in food microbiology but also underscore the potential of Eos particularly oregano as multifunctional agents in active food packaging strategies.

Testing the disinfectant effects of biodegradable oligomeric compounds

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Introduction. During the COVID-19 pandemic, the demand for disinfectants increased. However, their overuse and misuse also increased. This can lead to a worsening of the problem of antimicrobial resistance, but also to serious impacts on ecosystems and human health. An ideal disinfectant should be effective even at low concentrations, gentle on human health, and effective against a wide range of microorganisms. It should also be easily biodegradable, environmentally friendly, and cheap to produce, which could be achieved by lactic acid biopolymers. Nowadays, biopolymers are crucial mainly due to their origin from renewable sources and non-toxic nature. In a regular laboratory, the bacterial effectiveness of a disinfectant can be demonstrated using the method described in the ČSN EN 13727+A2 standard.

Methodology. The first step was to determine the minimum inhibitory concentration (MIC) using the dilution method in 24- well plates. The MIC was determined for four microorganisms – *Escherichia coli* (CCM 4517), *Staphylococcus aureus* (CCM 4516), *Pseudomonas aeruginosa* (CCM 7930), *Enterococcus hirae* (CCM 4533). Thirteen substituted lactyl-lactates synthesized at the Department of General and Inorganic Chemistry, University of Pardubice were tested in this way. Only one of them was further tested according to the ČSN EN 13727+A2 standard.

Results. The most promising sample was tested in this work according to the ČSN EN 13727+A2 standard in 1.5% and 2% concentration. The 2% concentration of the tested substance was not effective on *Enterococcus hirae*. At the same time, the sample was sent to the accredited laboratory EMPLA. There, according to the ČSN EN 13727+A2 standard, the tested substance met the requirements for reduction in 3% concentration.

Conclusion. The tested sample shows a bactericidal effect even at a low concentration (3%) compared to, for example, alcohol (30%) or povidone-iodine, which is typically produced in a concentration of 7.5-10%

Life on plastic: understanding fungal biodiversity on Mediterranean floating microplastics

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Microplastics (MPs) represent a threat to all natural environments due to their ability to enter the food chain and carry pathogens and pollutants. Each year approximately 630,000 tons of plastics penetrate in European sea, becoming eventually microplastics. On its surface a biofilm consisting of microorganisms (mainly algae, fungi and bacteria) is soon established, this ecological niche has been called "Plastisphere".

Investigating the fungal community of floating and sedimented MPs is essential to clarify the dynamics occurring in the plastisphere. Beside, marine fungal biodiversity is still largely unexplored and a culturomic approach is pivotal to discover and preserve taxa with biotechnological potential.

Samples of MPs were collected from 6 transects between the Livorno harbour and the protected area "Secche della Meloria" (Tuscany, Italy), using Manta nets. Seawater samples (SW) were also collected to compare the fungal communities of the two matrices. MPs were sonicated to detach microorganisms and then suspension obtained was plated on different media and incubated at 15°C. SW was filtered and filters were directly plated. Isolated fungi were identified through an integrated approach combining morphological observation and molecular and phylogenetic analyses.

The nearly 400 isolated fungi were ascribable to 98 taxa, of these 55 exclusively retrieved from MPs and only 21 (20% of the total) found in seawater. Indeed, MPs and SW mycobiota were different in terms of species composition and ecological traits. MPs community changes from the harbour to the open sea, as evidenced by the diversity indexes. Moreover, by the comparison with a recent paper (Florio Furno et al. 2022) we also found different communities on floating and sedimented MPs.

Preliminary observations suggest the presence of putative new taxa currently being studied. In the near future, strains isolated from MPs will be characterized for their potential degradation capabilities toward different xenobiotics and bioactive molecules production.

Advanced photocatalytic foam-based filters for microbial decontamination of air and water: a material-microbe interface study

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The growing demand for sustainable solutions in microbial decontamination of water and air has driven the development of novel photocatalytic systems. This work presents a multidisciplinary approach combining nanomaterials, interfacial chemistry, and microbial ecology, aimed at addressing microbial and chemical pollution in environmental matrices. The project focused on the synthesis of doped titania (TiO_2) nanoparticles (NPs), engineered for activation under UVA/visible light and integrated into solid foams and 3D-printed multiscale structures. These photocatalytic filters exhibiting enhanced surface area and hierarchical porosity, and designed to optimize microbial contact and photocatalytic activity, were included in a pilot scale plant. The system was tailored for both air and wastewater purification, specifically targeting pollutants and microbial contaminants from the agri-food sector. Photocatalytic experiments using showed a complete inactivation of reference microorganisms (both Gram-negative and Gram-positive bacteria and yeasts) under UV light. Antimicrobial efficacy of Al-N doped TiO_2 NPs was tested *in vitro* under different light exposures, with survival curves quantifying bactericidal performance. Following, tests using doped NPs-embedded foam filters were carried out, to possibly correlate material properties (zeta potential, BET surface area, interfacial viscoelasticity) with microbial inactivation efficiency. The photocatalytic and antimicrobial behaviour of the TiO_2 systems was closely linked to nanoparticle features such as size, crystalline phase, and dopant composition. The use of scalable and low-impact synthesis methods, including sol-gel and hydrothermal techniques with non-toxic precursors, supported the environmental compatibility of the approach. These findings underscore the importance of nanoparticle-interface interactions in microbial control strategies and highlight the relevance of engineered porous photocatalysts as eco-friendly alternatives to conventional treatments. This research contributes to bridging material innovation and microbial control, offering scalable, low-impact solutions for the circular management of water and air resources.

Characterization and assessment of bacteriological contamination by *Salmonella spp.* in several poultry farms in the Prefecture of Kindia (Republic of Guinea)

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Poultry farms are one of the main reservoirs of enterobacteria, particularly Salmonella, which are often associated with foodborne illnesses. The present study aims to characterize and evaluate the contamination of some farms by strains of *Salmonella spp*. in the Kindia Prefecture. Based on the surveys, two types of farms (Semi-modern and modern) were identified. A total of 66 samples, including 15 cloacal swabs and 51 fresh droppings, were collected between June and September 2024. *Salmonella spp*. strains were isolated according to ISO 6579-1:2017 and confirmed in Api 20E galleries along with other enterobacteria. These methods allowed the isolation of 27 germs, including 22 *Escherichia coli* (33.33%) and 4 *Salmonella spp*. (6.06%). Antibiotic sensitivity was determined by the diffusion method on agar media. Susceptibility testing results showed that 75% of isolated *Salmonella spp*. were resistant to Ampicillin (AMP10), Nitrofurantoin (F300) and Trimethoprim+Sulfamethoxazole (SXT25). This study highlighted the presence of *Salmonella spp*. in certain farms in Kindia prefecture and proved the need for increased surveillance to limit the spread of this germ in the area.

Hypogean wine cellars as source of new yeasts useful for development of biological tools addressed to increase the sustainability in winemaking

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The structure of the cellar might influence the characteristics of the wine produced in a given area. For example, the hypogean wine cellars can be considered production and aging sites of traditional wines, but these ecosystems could also be the keepers of an indigenous microflora, not contaminated by the use of commercial starters. The yeasts resident in these habitats might possess characteristics that enabled them to survive in such extreme environments. These new yeasts can be used for developing biological tools able to increase the sustainability and energy saving in wine production. This approach might be based on the selection of starter yeasts capable of maintaining good fermentative activity even in the absence of strict temperature control, thus reducing the energy consumption to maintain the optimal temperatures during fermentations. In this study, the cultivable yeast population sampled over two consecutive years from hypogean cellars was studied. Yeasts identified as Saccharomyces cerevisiae were characterized for traits potentially interesting in winemaking, such as the resistance to antimicrobial compounds (i.e. sulphur dioxide, …). Selected S. cerevisiae strains were tested in fermentations performed at temperatures above and below the optimum value for S. cerevisiae, by evaluating the sugar consumption rate, while the final wines were analyzed for the content of main aromatic compounds. These preliminary results confirm the high potentiality of unconventional habitat, such hypogean cellars, poorly investigated until now, as a source of new yeast starters which could represent a useful tool for wineries to increase the energy efficiency of the vinification process.

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Mycobiota monitoring for environmental sustainability: challenges, problems and solutions

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In recent years, the assessment of yeast diversity is taking advantage of culture-independent techniques, based on the use of total environmental DNA (more rarely RNA) for next generation sequencing of specific markers amplicons (metabarcoding, meta-taxonomy etc.) or if the whole DNA (shotgun or metagenomics). The two approaches are deeply different in several aspects, among which the amount of reads obtainable per locus or per gene. The whole scenario is further complicated by the presence of different sequencing platforms producing reads of different lengths. While long-read is theoretically preferable for the larger amount of information and for the reduced likelihood of chimeras inherent with short-reads alignments, the higher error rate and the lack of long-read databases currently reduce the possibilities offered by this approach1.

Various aspects of yeast genetics and genome arrangements impact metagenomics and must be considered for careful choice of the loci used for determining yeast diversity in different environments. Furthermore, the strong performances of these innovative techniques call for more insightful approaches to sampling, also in consideration of the high genetic plasticity of yeasts under stressful conditions. A number of technical issues, ranging from strategic choices to procedural details, affect the accuracy and reproducibility of results. Altogether, the yeast community should consider issuing guidelines in order to promote highly significant and reproducible studies that would be published according to specific standards for the various approaches. Furthermore, recent developments at the taxonomic level and the question of using sequences as types are hot questions now under active discussion, that will be presented and discussed.

Enrichment culture from a microbial community naturally living on spray painted surfaces for bioclening purpose

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Unwanted graffiti made by spray paints are one of the most severe pollutants that affect historical and modern buildings. Current cleaning protocols based on chemical or physical procedures are often not completely effective. Microorganisms offer a powerful, low-cost, safe and eco-friendly solution to remove graffiti from urban surfaces. However, a fully satisfying biocleaning procedure for spray paint has not yet been achieved due to the complexity of the paints. Spray paints contain xenobiotic compounds both as binders and pigments as well as heavy metals, creating an extreme environment for most microbial life. Microorganisms naturally living on spray paints have adapted to thrive in these harsh conditions and could be potential candidates. However, microorganisms suitable for biocleaning graffiti need to be cultivated in order to be available in high quantity at a low cost. In this research a lab-scale graffiti environment system was set up and an enrichment culture to retrieve the culturable endemic microbial community adapted to live on spray painted surfaces was obtained. A next generation sequencing approach was employed to obtain the molecular fingerprinting of the enriched microbial community structure living on graffiti spray paints and its trend over time. Finally, the most abundant bacterium was isolated and its 16S rRNA gene fragment was sequenced using standard methods.

Soil fungal strains degrading hexachlorocyclohexane isomers as potential bioresources for future mycoremediation applications

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Soil microbiota is increasingly threatened by human activities and global change, especially due to persistent chemical contamination. Hexachlorocyclohexane (HCH) isomers are globally recognized as hazardous and highly persistent organic contaminants. Thanks to their resilience and metabolic versatility, soil fungi represent promising bioresources for HCH-contaminated soil bioremediation. For this study, soil samples were collected from two plots in the National Priority Site "Bacino del Fiume Sacco" in the Metropolitan City of Rome, sampling up to 100 cm. Samples were then divided into topsoil (TS) and subsoil (SS). Microbial community-level physiological profiles were assessed by Biolog EcoPlate™, revealing differences in metabolic activity between TS and SS communities. The fungal fraction was further investigated by quantifying CFUs/g of dry soil. To isolate fungal HCH-degrading strains, a selective enrichment procedure was performed using a mixture of HCH-isomers as sole carbon source, from which resulted several fungal isolates, predominantly belonging to *Fusarium*, *Alternaria*, and *Acremonium*-like genera, now preserved in the Fungal Biodiversity Laboratory Culture Collection (Sapienza University of Rome). To individually assess isolates' ability to exploit HCH-isomers a multiwell assay was performed providing single isomers as sole carbon sources, revealing different activity depending on the isomer/isolate combination. Finally, the isolates were tested for biosurfactants production, as HCH-bioavailability enhancers. These results highlight the potential of selected fungal strains as candidates for future mycoremediation applications aimed at HCH-contaminated soils.

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Influence of diet on the microbial composition of *Tenebrio molitor* frass: implications for biofertilizer use

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Tenebrio molitor frass (TMF), composed of excreta, uneaten feed, and exuviae, is an emerging by-product of insect farming with potential application as a sustainable biofertilizer. Its nutrient and microbial content can influence soil fertility and plant health, with the insect's diet playing a key role in shaping the frass microbiota. However, limited data are available on the direct microbial composition of frass and its variation in response to dietary changes. In this study, mealworm larvae were reared on two diets: (A) wheat bran and feed yeast, and (B) the same base supplemented with brewer's spent grain. TMF samples were collected in triplicate at time 0 (baseline, before the onset of diet-specific effects), and subsequently at 30, 60, and 90 days. All samples were thermally treated at 70°C for 90 minutes in compliance with EU regulations. Samples were analyzed for total microbial counts and key microbial groups, including *Enterobacteriaceae, Pseudomonas spp., Enterococci*, lactic acid bacteria (LAB), *Clostridia*, and *Bacillus spp*. spores. Selected colonies were identified by 16S rRNA Sanger sequencing, and microbial communities were further profiled using full- length 16S rRNA amplicon sequencing (Oxford Nanopore Technologies, Epi2me wf-metagenomics pipeline). The culturomic approach revealed significant differences in microbial prevalence between diets, with diet B leading to higher loads of LAB, Clostridia, and spore-forming Bacillus spp. (reaching ~8 Log CFU/g).

Temporal variation in microbial composition was observed in both diets throughout the experimental period. Amplicon sequencing revealed a consistent dominance of *Enterococcaceae, Staphylococcaceae, Enterobacteriaceae, Streptococcaceae,* and *Bacillaceae*.

These findings highlight the pivotal role of diet in shaping frass microbiota, with potential implications for enhancing the agronomic value and safety of TMF as a biofertilizer in circular and sustainable agriculture systems.

Biodiversity of yeasts from grape berries collected in the Campania region using culture-dependent techniques

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Vineyards naturally support the proliferation of diverse microorganisms, providing a suitable habitat for their development. Microbial communities play a crucial role in grape cultivation and the winemaking process. For this reason, the study of yeast biodiversity on grape skins is a relevant topic in oenological microbiology, as various fungi and bacteria can directly affect vine health and the final wine, either beneficially or detrimentally. Since wine is a fermentation product, the vineyard is a significant entry point for microorganisms that influence wine quality. Native yeasts, particularly those initiating spontaneous fermentation, contribute to both the dynamics and aromatic complexity of the final product. Species such as *Hanseniaspora* and *Metschnikowia*, although not always dominant in alcoholic fermentation, can significantly impact the sensory profile of wines. This research investigated the yeast communities present on the skins of Barbera and Aglianico grape varieties from three wineries in the Campania region, comparing untreated grapes with those treated with a biostimulant. Yeasts were isolated using selective culture media and identified through molecular techniques. Various indices were applied to evaluate biodiversity. The results showed that *Aureobasidium, Metschnikowia*, and *Hanseniaspora spp*. were among the most frequently occurring genera. Species richness ranged from two to four species per sample. Overall, this type of study highlights how viticultural practices, including the use of biostimulants, can influence microbial ecology on grape surfaces. In this case, no significant differences were observed between the control and treated samples. Therefore, the application of the biostimulant did not appear to alter the microbial communities on the grape surface. However, these findings will be further deepened with next-generation sequencing (NGS) analysis. Biodiversity studies are essential for understanding the concept of terroir, demonstrating how the complexity of the natural environment and sustainable winemaking practices can promote the production of unique and high-quality wines.

Modulation of black soldier fly (*Hermetia illucens*) gut microbiota by agri-food processing by-products

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Gut microorganisms are involved in many different aspects of insect physiology such as nutrition and development: an imbalance in their composition can negatively affect insect health and performance. Insect farming has emerged as a promising approach for the valorization of agri-food processing by-products, enabling both waste reduction and the sustainable production of alternative protein sources. Among candidate insect species, the Black Soldier Fly (BSF, *Hermetia illucens*) has received particular interest due to its remarkable efficiency in bioconverting a wide range of organic substrates. Here, we investigated how different agri-food processing by-products influence both the gut microbial community and the performance of BSF larvae, and how the supplementation with probiotics could improve host growth and bioconversion. Larvae were reared on different organic substrates - brewer's spent grains, okara, potato peels and potato selection waste - alongside a standard laboratory diet. While BSF larvae were able to complete development on all tested substrates, performance metrics varied depending on the diet with a reduced larval survival on potato peels and potato selection waste. Gut bacterial composition and abundance were assessed by 16S rRNA high-throughput sequencing and quantitative PCR (qPCR), respectively. Our results revealed that distinct gut bacterial communities were hosted by larvae reared on different substrates. Then, we decided to apply bacterial probiotics to BSF larvae reared on the two suboptimal diets to observe any beneficial effects. In few cases the supplementation with bacteria suggested a positive effect. All in all, the characterization of the gut microbiota of insects provides valuable insights into the complex dynamics of insect–microbe interactions and supports the development of probiotic-assisted insect farming strategies.

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Diversity and composition of filamentous fungi communities isolated from Sardinian two-row barley and hydrophobins involved in primary gushing

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Fungal infections in barley and malt are a known cause of primary gushing in beer, a phenomenon whereby beer overflows vigorously and spontaneously from the package as soon as it is opened, without agitation.
Several studies have demonstrated that some filamentous fungi present on barley grown in fields produce hydrophobins, which are amphiphilic surface-active proteins. This study investigated the fungal ecology and activity in a two-row barley field and in barley and post-harvest barley grains and malt samples using plating methods, direct isolation, microscopic techniques, metagenomic sequencing on the Illumina platform, and mass spectrometry (MS)-based proteomics. The distribution of fungi was examined across three phenological stages of barley at an experimental field site in Sardinia. Over 100 fungi belonging to the genera *Alternaria*, *Aspergillus*, *Cladosporium*, *Fusarium*, *Mucor*, *Penicillium*, *Rhizopus* and *Trichoderma* were isolated. The secretome obtained from fungal cultures in a YNB medium was analysed using a proteomic approach. Hydrophobins were identified in the *Aspergillus* and *Trichoderma* genera, accounting for 25–30 % of the amphiphilic proteins detected. ITS rRNA gene sequencing and fungal secretome analysis enabled us to obtain preliminary results concerning adaptive changes in the fungal lifestyle. Further research is required to investigate the potential of hydrophobins as a marker for improving malt quality in breweries.

Optimizing biogas production through Machine Learning approaches on integrated microbial and process data of full-scale anaerobic digesters

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Anaerobic digestion (AD) in full-scale biogas plants is a complex and dynamic process shaped by operational conditions, microbial community structure, and the chemical profile of the substrate. Gaining a holistic understanding of these interdependencies is key to optimizing methane yield and improving the overall efficiency and sustainability of biogas production.

In this study, we conducted a systematic investigation across five full-scale biogas plants using cattle slurry alone, or pig slurry supplemented with agri-food byproducts as feedstock. A dataset was collected, comprising operational parameters and metadata recorded on-site, alongside digestate samples for microbial and physico-chemical analyses. Microbial communities were characterized through 16S rRNA gene amplicon sequencing, while volatile fatty acid (VFA) profiles were determined using gas chromatography technique.

All collected data were organized, pre-processed and exploit to train predictive models using advanced Machine Learning (ML) approaches. These models aimed to estimate energy output and biogas composition—specifically methane, carbon dioxide, and hydrogen sulfide concentrations—with high accuracy. Multiple regression models were trained and evaluated using varying combinations of input features, including process variables and microbial community profiles.

Results demonstrate that incorporating microbial data significantly enhances prediction accuracy, underscoring the critical role of microbial composition in AD performance. The findings highlight the potential for identifying key microbial biomarkers associated with process efficiency, paving the way for more intelligent, data-driven management of biogas systems.

Screening of oleaginous microorganisms as potential biofuel producers

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In the last decades, the scientific community and the international public opinion have shown increasing interest in lipid biofuels obtained from microbial biomass, considered a possible sustainable alternative to fossil fuels. The main aim of this research is the selection of promising strains among oleaginous yeasts and bacteria for a potential use in the energy industry. An initial screening was conducted on strains isolated from different food and environmental matrices known in literature for their lipidogenic potential. In particular, 15 yeast strains isolated from spoiled buffalo yogurt and Marastina grape surfaces, and 5 bacterial strains isolated from Montenegrin *Sudžuk* salami were selected. The yeast strains belong to the following species: *Saccharomyces cerevisiae, Yarrowia lipolytica, Rhodotorula babjevae, Rhodotorula glutinis, Pichia terricola*. The bacterial strains belong to the species: *Bacillus pumilus, Oceanobacillus kimchii, Staphylococcus pseudoxylosus, Bacillus aerius* and *Bacillus halotolerans*. Yeasts were grown in liquid medium at low temperatures (15°C), while bacteria were grown in high salinity conditions (3% NaCl), in order to determine by spectrophotometric measurements the time required for each culture to reach the late exponential phase, which is hypothesized as the time of maximum lipid synthesis, under the conditions identified as the stimulus for the activation of lipid biosynthetic pathways. Lipid production was evaluated by FTIR spectroscopy in terms of relative abundance/accumulation. Based on the results of the preliminary screening on lipid synthesis capacity, the strains *R. babjevae* (50) and *R. glutinis* (72) were the most promising strains and were analyzed by viable plate count and spectrophotometric analysis, in order to construct their growth curve and identify the optimal phase for maximum lipid biosynthesis, determined by FTIR analysis. This study represents a starting point for the selection and optimization of growth conditions of wild type oleaginous microorganisms that could be used in an industrial upscaling system.

Antimicrobial resistance inputs in agriculture settings: insights for a one health approach

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Biosolids and reclaimed water are valuable resources for reintroducing organic matter into agricultural soils and reducing the water footprint of intensive food production systems.
This work aimed at assessing Antibiotic-Resistant Bacteria (ARBs) and Antibiotic Resistance Genes (ARGs) inputs in agriculture settings under circular economy practices.
Resistome of 25 biosolid and 14 irrigation water samples under field conditions was characterized using shotgun metagenomic sequencing, followed by identification of antibiotic-resistant phenotypes of cultured samples and isolation of live ARBs followed by characterization through whole-genome sequencing.
The most represented resistance determinants in biosolid samples conferred resistance to daptomycin, followed by mupirocin, fusidic acid and bacitracin.
In water samples, the ARG profile was similar to that of biosolids, but with lower bacitracin resistance and higher polymyxin resistance determinants. Notably, beta-lactamases of all Amber classes were more abundant in water samples than in biosolids. We observed that metagenomic findings had correspondence with live bacteria isolated from the samples expressing the same antimicrobials' resistance.
ARGs have recently been recognized as emerging hazards in urban wastewater, the source of reclaimed water for agriculture. Despite treatment process, our data demonstrate that, in both biosolids and reclaimed water, the resistome persists and spreads to the resident microbial communities. This creates a reservoir of functional ARGs, potentially able to disrupt microbial ecosystems of agriculture soils and possibly reach animals and humans via feed and food chain.

The unappreciated importance of rhizobia in DMSP cycling

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Dimethylsulfoniopropionate (DMSP) is an abundant anti-stress molecule produced by marine algae, bacteria and some plants. Once released into the environment, DMSP is a major nutrient source, key in global carbon and sulfur cycling, and the main precursor for the climate-active gas dimethyl sulfide (DMS). Different marine microbes degrade DMSP to generate DMS by having diverse DMSP lyases. Hence, globally, most DMSP and DMS thought to produce by marine organism. However, many terrestrial plants produce DMSP and common soil bacteria of the rhizobia group can contain DMSP lyase enzymes. Since no studies focus on rhizobial DMSP cycling, we explored the biodiversity, distribution and importance of DMSP lyases in these symbiotic nitrogen-fixing bacteria.

From our bioinformatics analysis and functional validation by Gas chromatography (GC) we show that DMSP lyase genes are common in rhizobia with many containing functional DddD, DddL, family enzymes. Significantly, we identify and characterise a novel DMSP lyase enzyme in rhizobia, termed DddS. Finally, we show that the *ddd* genes are widespread and expressed in diverse terrestrial soils.

This data challenges the belief that DMSP cycling is strictly a marine process and highlight rhizobia as potentially important DMS producers in terrestrial DMSP cycling that might interact with DMSP-producing leguminous plants. The presence of the DMSP cycle in terrestrial systems highlights their potential contribution to cloud formation, alongside marine ecosystems, enhancing our knowledge of climate-related biogeochemical processes.

Grape stalks and olive pomace by-products in sustainable cows feeding: evidence from *in-vitro* rumen fermentation

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The agri-food system in Trentino includes significant wine and olive oil production. A promising opportunity for circularity lies in the reuse of grape stalks (GS) and olive pomace (OP) as feed supplements for dairy cows. Recent studies have shown that daily supplementation with GS and OP can enhance milk nutritional quality without compromising milk yield. Their high content of phenolic compounds, residual oils, and tannins may also positively influence rumen microbial communities. This study aimed to evaluate the effects of GS and OP on rumen microbiota modulation and methane (CH_4) emissions reduction using the in-vitro Rumen Simulation Technique (RUSITEC). Six diets were tested: a control (CTRL); grapeseed meal (GSM) at 8%; grape stalks from Lagrein (GSL), Cabernet Sauvignon (GSCS), and Merlot (GSM) at 8%; and olive pomace (OP) at 4% (all percentages on a dry matter basis). Two RUSITEC systems with six bioreactors each were used across three experimental runs (n = 6 per diet) and samples were collected over 5 days for analysis of fermentation parameters. No significant differences were observed between the CTRL and by-product diets in terms of pH, or protozoa counts, albeit redox potential was increased by OP compared to CTRL. Mean gas composition ranged from 14.0–15.8% for CH_4, 76–78% for CO_2, and 0.07–0.09% for H. A potential effect on reduced ammonia production was observed in GSCS ($P<0.05$), followed by GSL and OP diets ($P<0.10$) compared to CTRL, despite the similar crude protein degradation. Among volatile fatty acids, caproate was the most affected being decreased by several by-product diets, suggesting a shift in microbial activity, which will be further investigated using Illumina MiSeq sequencing. The data collected show promising trends in the use of agri-food by-products in sustainable ruminant feeding systems. Ongoing research will further explore these results assessing their practical applicability.

High-throughput profiling of bacterial communities in the gleba of the summer truffle (*Tuber aestivum* Vitt.): a comparative analysis

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Truffles are the fruiting bodies of hypogeous ascomycetes that form ectomycorrhizal associations with the roots of various plant species, including *Quercus* spp, *Corylus* spp, *Carpinus* spp, *Ostrya* spp and *Tilia* spp. Several species of truffle are highly prized for their distinctive aroma and flavour. However, the productivity of natural truffle grounds has been declining in recent years largely due to human activity and climate change.

The role of bacterial communities in truffle biology is increasingly recognised as an important area of research, as these microorganisms may influence multiple aspects of truffle ecology, including the establishment and functioning of ectomycorrhizal associations. Certain bacterial taxa, often referred to as 'mycorrhizal helper bacteria', have been proposed to facilitate the symbiosis between truffles and their host plants, potentially supporting nutrient dynamics and contributing to the productivity of truffle-associated ecosystems. Additionally, bacterial communities have been associated with the production of volatile organic compounds that contribute to truffle aroma. These volatiles may aid in attracting mycophagous animals responsible for spore dispersal, thereby potentially influencing truffle reproduction.

Ninety-six DNA samples were extracted from the gleba of summer truffles (*Tuber aestivum* Vitt.) collected in ten Italian regions (Apulia, Basilicata, Calabria, Campania, Emilia-Romagna, Lazio, Marche, Piedmont, Tuscany, and Umbria) and analysed to investigate the diversity of bacterial communities. Shotgun metagenomic and 16S rRNA gene amplicon sequencing were employed for this purpose. This dual approach is used to characterize the taxonomic composition of bacterial communities associated with the gleba of truffles from different regions of Italy, providing a foundation to explore their potential ecological roles in supporting truffle development, local adaptation, and contributions to aromatic traits.

Different microorganisms for bio-processing of lentil hulls, in view of their use as food ingredient

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Lentil hulls (LH) are by-products from handling of lentils, amounting at 20-30% of the total quantity of lentils subjected to dehulling. Although mainly used in animal feeding, they could be exploited as ingredients for food/dietary supplements, because of their content in proteins, lipids, vitamins, minerals, phenolics, and, above all, dietary fibre. However, LH contain antinutritional factors (ANF), such as phytate, saponins, tannins, and inhibitors of digestive enzymes. Since previous research showed that microorganisms could decrease the concentration of some ANF, during the OnFoods project, we aimed to investigate bioprocessing of LH using lactic acid bacteria (LAB), or fungi (either unicellular or filamentous). *Lactiplantibacillus plantarum* LPLDL, *Lactococcus lactis* VMO01, *Lacticaseibacillus casei* BGP 93, *Lacticaseibacillus rhamnosus* LR 4PD (all provided by Sacco Srl), *Furfurilactobacillus rossiae* LV-S3, and five *Pediococcus acidilactici* strains (all isolated from lentil hulls that had been provided by Terre di Altamura Srl) were singly inoculated, at cell density ranging from 6 to 7 log CFU/g, in a mixture (1:4) of lentil hulls (ground to a particle size of about 500 μm) and water (both previously autoclaved, separately). Fermentation was run at 30-37 °C for 48 h. Yeasts (*Debaryomyces hansenii* DSM 70238, *Geotrichum candidum* DSM 13629, *Wickerhamomyces anomalus* DSM 6766) and filamentous fungi (*Penicillium roqueforti* D1, *P. roqueforti* ITEM 9579, *Rhyzopus oryzae* ITEM 18876, belonging to Microbial ISPA-CNR Collection) were inoculated, at 7 log CFU/g, in an autoclaved mixture of LH and water. Solid state fermentation was run at 25-30 °C for 120 h. Overall, LAB increased by 1 to 2 log cycles during fermentation, decreasing pH to 4-5.4. L. plantarum LPLDL showed the best growth and acidification performances. All the fungal strains increased their cell density and marked proteolysis was found in LH bioprocessed by filamentous fungi. Levels of ANF were variously affected, depending on the microorganism used.

From waste to feed: fermentation of vegetable and dairy residues for sustainable Black Soldier Fly Larvae (BSFL) feed production.

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Black Soldier Fly is one of the most thoroughly studied insects, as an excellent source of low-impact protein, fats and chitin.
Vegetable and dairy industry residues represent an adequate nutrition for BSF larvae if they are supplied in the right proportions and in an excellent state of preservation. To maintain these wastes in an excellent state of preservation, guided microbial fermentation can be a low-cost resource, but it requires to be fine-tuned in relation to the specific substrates on which it is applied.
For this purpose, lactic acid bacteria (LAB) and yeasts seem to be the best candidates, both to reduce the perishability of waste-based feeds and to ensure the safety of the obtained products. LAB and yeasts are known to inhibit the growth of unwanted microorganisms by lowering the pH and producing antimicrobial factors. Yeasts, in particular, are also known to produce ethanol and for their detoxifying activity against mycotoxins.
In this project, we studied the qualitative and quantitative composition of the indigenous microflora of two test feeds designed based on the nutritional exigences of BSFL and composed of Winter Vegetables Waste plus Whey (WVW+W), and Summer Vegetables Waste plus Whey (SVW+W). Our work demonstrates that a simple spontaneous pre-fermentation of two days at a temperature of 27 ± 3°C is enough to significantly reduce the load of Enterobacteriaceae in both feeds. Lower significant reduction was observed against molds. The dominant LAB and yeasts, spontaneously present in the two matrices were isolated and characterized both at phenotypic and molecular level.
An opportune selection of LAB and yeasts, isolated from waste-based feeds, will be lyophilized to obtain a ready-to-use freeze-dried microbial starter, able to stabilize a waste-based feed for black soldier fly larvae (BSFL).

Exploring *Starmerella bacillaris* as a sustainable biocontrol strategy against *Botrytis cinerea* in vineyards

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Increasing awareness of the environmental and health risks associated with chemical pesticides has highlighted the need to reduce their use in the agri-food sector, stimulating the development of alternative control strategies. Pre-harvest management represents a critical stage for implementing measures that limit pesticide residues in food products reaching consumers. Among the most promising alternatives to chemical fungicides is the use of microorganisms as biocontrol agents.

This study investigated the potential biocontrol activity of the yeast *Starmerella bacillaris* applied as a vineyard treatment against the spoilage fungus *Botrytis cinerea*.

Applications of *S. bacillaris* were carried out on Pinot grigio grapes at three key phenological stages: pre- bunch closure, veraison, and pre-harvest. Quantitative PCR (qPCR) was used to monitor population trends of *S. bacillaris* and *B. cinerea* on the grape surface. Following each application, *S. bacillaris* established an initial population approximately one to two logarithmic units lower than the concentration of the sprayed yeast culture, followed by a gradual decline over time. In untreated grape bunches, *B. cinerea* growth was evident, whereas it was undetectable in those treated with *S. bacillaris*. Conversely, conventional antibotrytic treatments resulted in only a modest reduction in *B. cinerea* levels.

Metabarcoding analysis of the whole ITS region on grape surface fungal communities further supported the biocontrol effect of *S. bacillaris*. Additionally, *S. bacillaris* persisted into the grape juice obtained from treated grapes, and wines produced from this grape juice showed increased glycerol levels and comparable acetic acid content relative to controls, supporting the known positive contributions of this yeast during fermentation.

Overall, the findings underscore the potential of *S. bacillaris* as a sustainable alternative for gray mold management in vineyards, with the added advantage of enhancing wine quality.

Targeted culturomic approach to select cellulolytic bacteria using *Hermetia illucens* larvae as natural ecosystem

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The growing demand for sustainable resources led biotechnology research to use innovative approaches to transform lignocellulosic waste through bio-based technologies. *Hermetia illucens* larvae were used to isolate cellulolytic strains from their gut for biomass bioconversion, following a Canapule-based diet and evaluating its effect on midgut, hindgut, and the larvae–substrate mixture bacterial communities, using high-throughput sequencing and qPCR targeting the *bcs*Z gene (endo- 1,4-β-D-glucanase). Dietary changes led to significant shifts in hindgut bacteria compared to larvae fed an optimal (chicken feed) diet. Based on these findings, a targeted culturomics approach was performed using rearing substrate and gut samples from Canapule-fed larvae. Two enrichment media (A1: CMC-based; A2: *Arundo donax*-based; both with soil, larval extract, and hemp powder) were sampled at inoculation, and after 7 and 14 days at 30°C. Metagenomic analysis of enrichment cultures showed sampling time influenced bacterial composition. Substrates inoculated with gut showed increased *bcs*Z gene presence.

To isolate cellulolytic microorganisms, enrichment samples were plated on solid media with CMC, Avicel, *A. donax*, or Canapule powder. After five days at 30°C, plates were stained with 1% Congo red to detect cellulose-degrading bacteria. 417 bacterial isolates were grouped into 58 phenotypes by MALDI-TOF, ~32% of which showed cellulolytic activity. Among producers, 28 strains (halos ≥15 mm) were further screened: 86% showed exo-cellulase, 36% xylanase, 43% β-glucosidase, and 29% pectinase activity. *Pseudomonas phenolilytica* AV144.1, selected for enzyme production in liquid media with 1% CMC, yielded up to 0.27 U/mL endo-1,4-β-D-glucanase within 11 hours at 37°C in MiniBio reactors (Applikon), confirming its biotechnological potential. These findings validate the enrichment strategy and position *H. illucens* as a promising source of cellulose-degrading bacteria for bioconversion.

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In-vitro screening of different natural substances as control tools against *Plenodomus tracheiphilus*

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Mal secco of citrus plants, caused by the fungus *Plenodomus tracheiphilus*, is one of the most significant diseases affecting cultivated citrus trees. Despite the increased availability of antifungal agents in recent years, the treatment of fungal infections remains challenging for this disease. Essential oils (EO) or natural extracts, known for their antimicrobial properties, represent a promising alternative strategy. The objective of this study was to evaluate the antifungal activity of different natural compounds against *P. tracheiphilus* PT-Salv isolated from affected plants located in Acireale (Catania, Sicily). The tested compounds included 13 EO-based nanoformulations, 10 extracts obtained from agri-food by-products, and 3 botanical extracts. The antifungal efficacy of these compounds was assessed using the disc diffusion method. A mycelial plug was placed in the center of a PDA plate and the different dilutions of samples were imbibed in sterile cellulose discs (Ø 6 mm). Plates were incubated at 25°C for 20 days and fungal growth was assessed by measuring the mycelial diameter at 10 and 20 days. Cinnamon, garlic, mint, mustard, geranium, and thyme EO-based nanoformulations showed a total *in-vitro* inhibition of mycelial growth. Among these formulations, cinnamon and thyme exhibited the greatest antifungal effects at different concentrations.

Specifically, cinnamon and thyme formulations totally inhibited mycelial growth with first dilution (1:2) while a moderate growth was observed in other dilutions (1:4 and 1:8) compared to the control. Conversely, the other tested extracts did not show any inhibitory effect against the fungus. These results support the potential use of EOs as sustainable tools for controlling *P. tracheiphilus* and provide a solid foundation for further research for managing mal secco disease.

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Bioconversion of second cheese whey through mixotrophic cultivation of *Tetraselmis chuii*

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Scotta, or second cheese whey (SCW), is a lactose-rich by-product of ricotta production, typically considered as a waste. This study investigated SCW valorization through mixotrophic cultivation of *Tetraselmis chuii*, aiming for sustainable biomass production within a circular economy framework. The microalga was grown for 10 days in medium with 2.0%, 3.5%, and 5.0% lactose under controlled conditions. Additional trials with glucose/galactose tested the efficiency of hydrolyzed lactose uptake. Lactose- supported cultures achieved a 1.84-fold increase in biomass over photoautotrophic control. *T. chuii* was then cultured in 100%, 75%, and 50% SCW using both preadapted and non-preadapted inocula. Preadapted cultures at 100% and 75% SCW showed biomass increases of 1.74-fold and 1.52-fold over controls, respectively. Nutrient analysis revealed significant reductions in nitrates, phosphates, carbohydrates, and
non-purgeable organic carbon. These findings highlight SCW's potential as a low-cost substrate for microalgal cultivation and demonstrate the robust adaptability of *T. chuii* under mixotrophic conditions, particularly with preadaptation.

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Green antimicrobial revival: essential oils as sustainable antibiotic adjuvants in resistant foodborne pathogens

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Antimicrobial resistance (AMR) is an escalating threat to public health and food safety, undermining the efficacy of conventional treatments and hindering the control of foodborne pathogens. This study investigates the potential of *Thymbra capitata* L. (Cav.), *Corydothymus capitatus*, and *Thymus serpyllum* essential oils (EOs) as a natural approach to restore antimicrobial susceptibility in multidrug-resistant *Listeria monocytogenes* and *Salmonella enterica.*

The experimental design included the use of EOs in combination with conventional antibiotics, as well as the pre-exposure to sublethal EO concentrations against clinical and food-derived multidrug-resistant strains. Minimum Inhibitory Concentration (MIC) and Fractional Inhibitory Concentration Index (FICI) were determined, along with cell growth dynamics, to assess the antimicrobial activity of the antibiotic-EO combined treatments.

Results showed that sublethal EO concentrations extended the lag phase of *L. monocytogenes* by approximately 14 to 22 hours, reducing growth parameters and enhancing susceptibility to lithium chloride, nalidixic acid, vancomycin, and lincomycin. Exposure to *Thymbra capitata* EO also led to a reduction in ampicillin, gentamicin, and penicillin G MIC values in *L. monocytogenes* up to seven-fold (from 8 to 1 μg/mL) while, in *S. enterica*, EO–tetracycline combinations lowered MIC values from 256 to 4 μg/mL. As a results, EOs were able to reduce the MIC values of conventional antibiotics below the clinical breakpoints defined by international guidelines for *L. monocytogenes* and *S. enterica* (EUCAST, 2024; CLSI, 2020), thus restoring antimicrobial efficacy. EOs interact with multiple bacterial cellular targets, damage membrane integrity depleting the available ATP and therefore increase the antibiotic uptake by the cell, improving their effectiveness. These findings support EOs as multifunctional agents that modulate antibiotic efficacy and overcome AMR, providing a sustainable strategy to improve therapies and ensure food safety.

What can the current global bacterial taxonomy knowledge reveal about the actual (past and present) ecological success of each lineage?

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Up-to-date sources of man-known microbiology include GTDB https://gtdb.ecogenomic.org/, as the most complete repository (107235 species). Such dataset is hereby used as checklist of current taxonomical notions. In the mathematical 'zero assumption' of equal opportunity chance for each taxon throughout evolution, the 'perfectly even' community would have had today, within each of the 196 phyla, 2,8 classes, 9,5 orders, 24,9 families, 117,9 genera, and 547,1 species. The deltas between these set-point values and the truly observed and described numbers for each lineage member featured by the database, (numbers of species per genus, genera per family, families per order, orders per class and classes per phylum) allow to reveal: 1) What is the actually occurred uneven dynamics of evolutionary differentiation across each lineage?; 2) Which taxa are, at present, the 'winners' of the evolutionary race?, and have they always led the run, or when did they overtake their formerly faster competitors? 3) Are there chronological bottlenecks that violate the assumption of either a constant or of a progressive speed of evolution (i.e. is speciation to each deeper, and more 'modern' rank, consistently faster than its former step or is there evidence of evolutionary congestion 'jams'?); 4) which past age turns out to have been the most determinant to shape the modern assemblages? Which ones, among the taxa dominating today's diversity, have risen up just recently (e.g. by a radiation from genera, and which ones had instead started or accomplished the critical expansion that still keeps them as leaders, in remote times (e.g. by class or order radiations?; 5) Which patterns characterize in this respect the lineages of the most relevant bacterial agents in food, soil, industrial, clinical and environmental microbiology? Corresponding queries are addressed for the 5869 Archaeal species. Methods and results of this database-mined analysis will be presented and discussed.

Biofilm development and antifungal susceptibility in *Candida parapsilosis*: a comparative study of clinical and environmental strains

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Candida parapsilosis is a significant opportunistic pathogen associated with nosocomial infections, particularly in immunocompromised patients. This yeast species is known for its ability to form biofilms on medical devices, contributing to its persistence and resistance to treatment. The rising incidence of *Candida* infections underscores the need for novel antifungal agents. This study presents a comprehensive molecular and phenotypic analysis of 70 nosocomial and 30 environmental strains of *C. parapsilosis*, with a focus on their resistance profiles to the emerging antifungal agent Rezafungin, in comparison to two commonly used antifungals: Micafungin and Liposomal Amphotericin B. Molecular characterization was performed by sequencing the ITS and LSU markers to identify strain-specific variations. Phenotypic characterization involved assessing biofilm formation and antifungal susceptibility in both planktonic and sessile cells. Planktonic cells were evaluated using EUCAST standard broth microdilution methods, while biofilm assays were conducted to determine the sessile minimum inhibitory concentrations (SMIC). Our results showed no significant genetic diversity between the nosocomial and environmental *C. parapsilosis* strains. However, phenotypic analysis revealed distinct profiles for biofilm formation across the isolates. The antifungal resistance profiles of *C. parapsilosis* to the three agents tested were not significantly influenced by the origin of the isolates—whether clinical or environmental. These findings highlight the challenge of treating infections caused by this species, which is commonly found in the environment and exhibits comparable resistance profiles across different sources.

Functional potential of soil microbial communities in urban lawns under mowing and pollution pressure

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Urban ecosystems are complex environments shaped by both anthropogenic pressures and land management practices. In our study, we explored how mowing frequency and proximity to pollution sources affect the functional potential of soil microbial communities in roadside lawns. This investigation, part of a broader research project on sustainable urban greenery management in Katowice and Cracow, Poland, aims to find strategies that enhance urban ecosystem resilience through microbiological insight. Using predicted functional profiling based on data obtained using the short-reads sequencing of 16S rRNA, we analyzed microbial communities from lawns exposed to different mowing regimes and pollution levels. Lawns frequently mowed (six times per season) harbored bacterial populations enriched in metabolic pathways related to the degradation of plant-derived compounds indicating microbial adaptation to environments saturated with decomposing plant biomass and secondary metabolites. Moreover, these lawns also showed increased gene abundance in sulfur and nitrogen metabolism, likely triggered by constant input of plant residues due to frequent cutting. However, the most pronounced differences in functional potential were linked to pollution levels, particularly in lawns adjacent to high-traffic roads. These soils supported microbial communities rich in pathways for degrading xenobiotics and environmental contaminants, such as toluene, phthalates, styrene, 4-nitrophenol, and 4-nitrotoluene, as well as resistance to heavy metals. The prevalence of such pathways demonstrates microbial adaptation to urban chemical stressors and highlights the detoxification role of soil bacteria in polluted urban settings. These findings underscore the critical ecological role of soil microbiomes in urban green infrastructure. While mowing practices influence microbial metabolism primarily through organic input, environmental contamination exerts a dominant selective pressure shaping functional diversity. Recognizing and harnessing these microbial capabilities can guide urban ecological transition efforts. Our results contribute to the development of evidence-based maintenance guidelines for roadside lawns, promoting soil health, pollution mitigation, and ecosystem service enhancement in cities.

Field and postharvest detection and isolation of potential mycotoxigenic fungi in carob (*Ceratonia siliqua* L.)

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Ceratonia siliqua L. is an evergreen tree (Fabaceae family) mainly cultivated in the Mediterranean basin. Carob fruit (pods) rich in nutrients, are increasingly valued in food, pharmaceutical, cosmetic and livestock sectors. Pods flours are appreciated as food stabilizers, thickeners, and emulsifiers (E410) in the production of beverages and desserts, thus requiring high hygiene standards. Preharvest fungal infections and inadequate storage conditions of infected pods could lead to mycotoxins contamination of derivatives with significant health risks. This study aimed to identify potential mycotoxigenic fungi in field and during postharvest pods storage. Field evaluation was conducted on leaf and pod samples of Giubiliana, Excelsior, Racemosa, Nicols, and Amele di Bari cultivars from CREA-OFA germplasm. Pod samples were collected from three piles stratifications in a local warehouse, corresponding to 18 (S18), 12 (S12), and 0 (S0) months of storage. Total mesophilic microbial load was assessed using standard microbiological methods; molds were identified by macroscopic features (mycelium color and shape), and through microscopic observation of vegetative and reproductive structures. Field results showed significant microbial load variability among cultivars, highest in Giubiliana and Amele di Bari (6.2-6.5 log CFU/mL) lowest in Nicols (5.0 log CFU/mL), which also showed the least yeast and mold presence. In stored pods, *Aspergillus*, *Cladosporium*, and *Penicillium* genera were isolated in some samples, thus molecular identification at species level will be carried out. These results highlight that carob pods, both in the field and during storage, are exposed to infection by potentially mycotoxigenic fungal pathogens, which can be transferred to the final product obtained from pulp and seed grinding. Therefore, appropriate processing protocols are essential to preserve the final product quality and safety.

Sustainable bioplastic production from agrifood waste using *Cupriavidus necator*

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The increasing awareness of the scarcity of natural resources and the necessity to mitigate the ecological impact of plastic waste has led to a significant search for environmentally sustainable plastic materials. Biopolymers offer a dual benefit in the sustainable development field, being able to both preserve fossil resources and reduce carbon dioxide emissions. In this context, polyhydroxyalkanoates (PHAs) have garnered significant attention as a promising class of bioplastics due to their versatility. These natural polymers are produced through microbial fermentation of carbon sources and are stored as an energy reserve in the cytoplasm and inner membrane in the form of granules. In this study, wasted bread, potatoes and artichokes by-products were used as substrates for bioplastic production using *Cupriavidus necator* DSM428. Such matrices contain complex polysaccharides (e.g., starch in bread and potatoes, inulin in artichokes). Hence, to release fermentable sugars, biomasses were subjected to enzymatic or chemical hydrolysis. Enzymatic hydrolysis of bread waste yielded glucose in amounts of 142g/L, while the acid hydrolysis of potato and artichoke waste released 58g/L of glucose and 15g/L of fructose, respectively. Saccharides from bread, potato, and artichoke food wastes (glucose 25g/L, fructose 15g/L) were used to cultivate *C. necator*, yielding PHB contents of 24%, 32%, and 26%. When agri-food by-product hydrolysates (5g/L sugars) were combined with oil extracted from coffee grounds (EOC, 1.5%, v/v), PHB yields increased to 37% (bread), 47% (potato), and 45% (artichoke). PHB molecular weight and polydispersity index are fully comparable with data collected by previous studies. However, EOC significantly enhanced microorganism growth and PHB accumulation. These results demonstrate the feasibility of using various food wastes as substrates for PHB production by *C. necator*. Ongoing research aims to optimize the process using EOC as sole nutrient source and scale up the most effective conditions in a pilot bioreactor.

What fungi can do on soil: from bioremediation to stability

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Fungi and soil have a very complex and interdependent relationship. The functions of fungi as biodegraders of organic material, as fundamental symbionts at the rhizosphere level, as controllers of plant and animal pathogens are well known. The use of fungi as natural base solutions (NbS) has started to be of great potential. Among the main challenges for which fungi are under the research lens is the possibility that fungi may be useful for the recovery of soil, which has been subject to strong impoverishment and degradation, in the last fifty years. Hydrocarbons, heavy metals and plastics are certainly the main soil contaminants, directly effecting the agriculture and the health and safety of its products. No less important is the process of soil erosion which influences, among other things, its stability which is so important for agricultural production. What fungi can do for enhancing soil recovery and resilience? In this work, the main results obtained using fungal strains in soil bioremediation processes and on soil stability are reported. Strains involved in this research belong to four species: *Trichoderma asperellum, Penicillium chrysogenum, Aspergillus tubingensis, Fusarium oxysporum*. Analyses have been carried out on the effects of fungal activity on the main pollutants and on the some proprieties of soil such as hydromechanics, cohesion and compressibility. Results have indicated that fungal treatment have led to an improvement of soil health, and structure.

Nanoformulations for in-vitro and *in-vivo* antifungal activity against *Plenodomus tracheiphilus*

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Plenodomus tracheiphilus is a vascular pathogen that poses a serious threat to the citrus industry due to its economic impact and risk of spreading to disease-free regions. Disease management is particularly challenging, as no resistant lemon cultivars with acceptable agronomic traits are currently available. Control efforts are further limited by the high susceptibility of commercial varieties, the absence of authorized systemic fungicides, and increasing restrictions on copper-based products. In this context, the present study investigates the antifungal efficacy of cinnamon and thyme essential oils (EO)-based nanoformulations previously selected for their promising bioactivity against *Plenodomus tracheiphilus* (PT-Salv) isolated from affected plants located in Acireale (Catania, Sicily). Gas Chromatography-Mass Spectrometry and Dynamic Light Scattering analyses were used to evaluate the EO chemical composition and the physical properties of the developed formulations. The inhibitory effects of these nanoformulations were evaluated *in-vitro* on mycelial growth using the disk diffusion method at different concentrations. Furthermore, the minimal non- phytotoxic concentration was assessed trough leaf-spraying in planta on *Citrus aurantium* (sour orange) and *Citrus limon* (lemon) "Zagara Bianca" and "Femminello Continella", from a germplasm collection field of CREA. The results showed that both nanoformulations exhibited complete in-vitro inhibitory activity against *P. tracheiphilus* even to 1:2 dilution. Fungal growth was observed at higher dilutions (1:4 and 1:8), although it remained lower than in the untreated control. Regarding phytotoxicity, no adverse effects were observed on *Citrus aurantium* at any of the tested dilutions. However, phytotoxic effects resulting in leaf necrosis were highlighted only on lemon when formulations were applied undiluted. In conclusion, these first results suggest that the nanoformulations can be considered a potential sustainable tool for *P. tracheiphilus* infection management.

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Micropollutants are a problem, but the fungal kingdom can help to solve it

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Life on earth depends on freshwater, but this resource is more and more under pressure. Continuous discharge of polluted wastewater can cause significant environmental impacts on surface and groundwater, mainly due to the so-called Contaminants of Emerging Concern (CECs). Europe is taking action, with proposal aiming at the "polluter pays principle", forcing pharma and cosmetic companies to cover the extra costs to clean urban wastewater. Wastewaters treatment plant, and especially their sludge, are an accumulation point of micropollutants, causing their complex microbial community to adapt. SusWater project aims to study this biodiversity and its powerful enzymatic cascade. The fungal community of the contaminated sludge was studied using liquid enrichment technique, using CECs commonly found in wastewaters treatment plants. Overall, 104 fungi were isolated, mostly belonging to the Ascomycota phylum. Surprisingly Basidiomycetes (9%) and Mucoromycetes (7%) were quite abundant. The isolated fungi were tested for their capabilities to degrade CECs. The removal efficiency was evaluated with a proper HPLC-UV detection method. Fungi isolated from this extreme environment must have developed peculiar metabolic skills and were indeed capable of degrading the target CECs. Notably, the degradation yields were strain dependent, but also CECs dependent. For instance, fungi easily attacked estriol and diclofenac, while fungi were almost ineffective against carbamazepine. Overall, 30% of fungi halved the concentration of one or more CECs with few strains capable of removing more than 70% of the CECs. The two best performing strains are currently under study to evaluate their strength in a more complex systems, where the transformation of CECs is followed in real wastewaters. Studies are also ongoing to study the transformation metabolites and to integrate these catalysts in intriguing technological solutions as hydrogels.

Session 5

Scaling up microbial solutions for the industry

Food innovation for good: Scaling tradition

Stefan Cappelle

Puratos, Belgium

From ancient kitchens to cutting-edge labs, the story of sourdough is one of resilience, science, and flavor. Centuries of artisanal tradition meet the power of modern food science. Sourdough—a practice dating back thousands of years—was once nearly forgotten in the rush of industrial baking using baker's yeast.

But today, thanks to scientific breakthroughs and global collaboration, it's experiencing a spectacular revival. You'll explore how microbial diversity, local raw materials, and environmental nuances gave rise to unique sourdoughs.

You'll get an exclusive look into the world's only Sourdough Library, where traditions are preserved and decoded with the help of pioneering research from Prof. Gobbetti and Prof. Di Cagno.

Discover how these insights are fueling next-gen fermentation: robust starter cultures, custom microbial consortia, and health-boosting benefits like improved digestibility and postbiotic effects. Learn how Puratos is producing consistent, ready-to-use sourdough in 15 countries—scaling up without losing soul.

Whether you're passionate about baking, fermentation, health, or sustainability, this is not just a talk—it's an invitation to taste the future, rooted in the past.

Your view on bread will never be the same.

A molecular method for rapid detection of a *Metschnikowia pulcherrima* strain during grapevine biocontrol in industrial withering conditions

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Microbial biocontrol is a biological strategy receiving increasing interest as a sustainable innovation, effective for preventing and managing crop diseases and food spoilage arising from microbial contamination. The work hereby presented was carried out within a research project focusing on bio- protection against *Botrytis cinerea*, in the context of post-harvest biocontrol on fruits. Among biocontrol agents, non-*Saccharomyces* yeasts have recently gained attention in the wine industry for their ability to enhance organoleptic characteristics and, at the same time, for their bioprotective activity which can reduce the need for the use of chemical preservatives. A species with great potential as a bioprotective agent is *Metschnikowia pulcherrima*, which was proven to inhibit the growth of spoilage microorganisms through various mechanisms. In this work, a specific strain of *M. pulcherrima*, previously selected for its potential to inhibit *B. cinerea*, was tested in post-harvest conditions. Its ability to persist throughout the withering period of grapes for *Passito* wine production has been investigated for the inhibition of *B. cinerea* in real-winemaking conditions (industrial-scale withering). In order to rapidly assess persistence during withering via PCR, a pair of strain-specific primers was designed to differentiate the experimental strain from other strains of *Metschnikowia*, possibly present in the environment since the *M. pulcherrima* clade is common in the winemaking environment. The proposed molecular method, indeed, consists of a rapid technique based on an optimized protocol of colony-PCR with primers designed through in silico comparison of different nucleotide sequences of a specific region that allows differentiation with the type strain of the same species and other relevant strains of closely related species due to a SNP. The application of the strain-specific PCR showed that the strain applied persists on grapes throughout all the withering period, moreover its ability to inhibit *B. cinerea* was confirmed compared with the untreated control.

From waste to bioplastics: integrated approaches for polyhydroxyalkanoates (PHAs) production using wild type and engineered microorganisms

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PHAs are fully biodegradable and biocompatible polyesters synthesized by certain microorganisms as intracellular carbon and energy storage granules. Due to their plastic-like properties, they are considered a promising alternative to fossil plastics. However, their broader commercial use is hindered by high production costs, primarily due to the expensive carbon feedstocks and the process conditions required for microbial fermentation. To address these constraints, efforts have focused on utilizing cheap and readily available by-products, such as food and agricultural residues, as alternative carbon sources. A key challenge is the lack of naturally occurring microbial strains capable of efficiently metabolizing these complex substrates and producing PHAs in high yields. To overcome this limitation, the Waste to Bioproducts lab at DAFNAE explored several innovative strategies, including: i) the genetic engineering of *Cupriavidus necator* DSM545, one of the most effective PHAs-producing strains, to enable utilization of cheap substrates such as lactose-, lipid-rich or starchy residues. the enzymatic pre-treatment of substrates, such as applying galactosidase to whey. ii) the exploitation of anaerobic digestion for substrate pre-treatment to convert waste biomass into volatile fatty acids as well as CO_2, which served as effective carbon sources for PHAs synthesis by *C. necator* DSM545. In addition, to avoid the high costs linked to sterile fermentations, recent research has turned to extremophile microorganisms as promising alternative PHAs accumulators. These organisms thrive in extreme environmental conditions, offering a natural barrier against contamination and reducing the need for stringent sterilization. This approach seeks to merge the valorization of nutrient-rich agricultural waste, such as distilled wine lees, with the unique metabolic capabilities of extremophiles, developing a sustainable and economically feasible process for PHAs production. Although further research is needed to optimize PHAs yields, the approaches described offer promising pathways for industrial-scale PHA production and contribute to more sustainable waste management strategies.

Microbial biodiversity of traditional cheeses and natural dairy environments as drivers of food innovation

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In this study, we focused on enhancing the microbial biodiversity that characterizes traditional dairy productions and environments as a key driver for food innovation.
As part of an extensive sampling effort, including raw milk, curds, natural whey cultures, and different cheeses at different ripening stage or cold storage, more than 140 microbial isolates were preliminarily evaluated, resulting in a collection of 98 bacterial strains. Among these, 28 strains were selected for their possible application in milk processing. The research for technological traits in strains, belonging to *Streptococcus thermophilus*, *Enterococcus faecalis*, *Ent. gallinarum*, *Lacticaseibacillus paracasei*, *Limosilactibacillus fermentum*, *Lactococcus garviae*, *Lact. lactis* species, led to the identification of 3 strains with high production of γ-aminobutyric acid (GABA). In parallel, microbial biodiversity related to yeasts and filamentous fungi, allowed the collection of 168 strains. Proteolytic and lipolytic activities as well as the ability to grow on sugar-free and minimal medium "cheese like" substrates interested 19 non-toxigenic Penicillium strains. Competitive interaction assays against a ochratoxigenic *Aspergillus westerdijkiae* strain from cheese surfaces, guided the selection of promising strains from *P. charlesii*, *P. copticola*, *P. solitum*, *P. canescens*, *P. flavigenum* and *P. biforme* species. These strains demonstrated the potentiality to control the growth of the toxigenic species, thereby contributing to the safety of the ripening process while preserving the typical characteristics of the cheese and its deep connection to the traditional natural environment. Throughout the exploitation of microbial biodiversity from dairy samples, a GABA-producing bacterial starter and a fungal formulate for the secondary ripening of long-aged pasta filata cheeses were defined. Consequently, the production of a new functional cheese and the improvement of the secondary ripening of Caciocavallo cheese in natural caves were carried out.

Sequential anaerobic–aerobic bioremediation of chlorinated ethenes in a complex contaminated site

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In Europe, approximately 650,000 sites require remediation (2,600 in Italy), with chlorinated ethenes among the most widespread pollutants, detected in 10% of contaminated groundwater. Anaerobic degradation of highly chlorinated ethenes (tetrachloroethylene and trichloroethylene) results in the accumulation of cis-1,2- dichloroethylene and vinyl chloride, which are more efficiently degraded under aerobic conditions. A large-scale bioremediation project is implemented at a 33-hectare site affected by a petrochemical landfill, where chlorinated ethenes in groundwater exceeded legal limits by several orders of magnitude. The remediation strategy employs two sequential anaerobic and aerobic biobarriers perpendicular to groundwater flow. Bio-based substrates were injected to stimulate in situ microbial activity, with performance assessed through hydrochemical and microbiological monitoring over a two-year period.

Groundwater was collected from piezometers located in northern and southern transects over the landfill, and the anaerobic and aerobic barriers. Illumina 16S rRNA sequencing and qPCR targeting total bacteria, archaea, *Dehalococcoides*, *Dehalogenimonas*, and functional biomarkers, confirmed active reductive dechlorination in the anaerobic zone and vinyl chloride degradation in the aerobic zone. The northern transect exhibited higher relative abundances of *Dehalogenimonas*, *Desulfitobacterium*, and *Desulfuromonas*, along with high presence of functional biomarkers and greater degradation efficiency. GC-MS analysis of anaerobic microcosms set up with groundwater confirmed the microbial degradation of chlorinated ethenes, highlighting different efficiencies in the two transects. Ongoing microbial network analyses will assess potential competition between reductive dehalogenating bacteria, iron- and sulfate- reducing bacteria, and methanogens. These outcomes upgrade our understanding of microbial dynamics involved in bioremediation, reducing site uncertainties, informing remediation decisions and improving overall bioremediation efficiency.

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From lab to industry: scalable production of alkaline proteases from *Bacillus licheniformis* for sustainable biostimulant development

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One of the most significant strategies for the development of sustainable biostimulants is microbial extracellular alkaline proteases (EAPs) production. In the course of the SPINN project, 60 bacterial strains were isolated from different alkaline substrates using culture-dependent methods. From a first qualitative screening, 21 were selected based on their proteolytic activity, and the SPINN5 strain, molecularly identified later as *Bacillus licheniformis*, exhibited the highest enzymatic activity. The chosen strain was used to optimise the fermentative process for the synthesis of AEPs, modifying important variables such as pH, temperature, and media composition at the laboratory scale. We carried out parameter modelling using the Response Surface Methodology (RSM) analysis, which enabled us to automate the creation of analytical models helpful for achieving optimal bioprocesses. Optimisation of the initial process was also carried out in 5 L bench-scale bioreactors with systematic reduction in key culture parameters to yield maximum EAP production. SPINN5 grew and secreted protease best in a modified BMSM medium, under extremely alkaline conditions (pH 9–10) and in a temperature of 37°C to 45°C. Under optimised conditions, SPINN5 showed the highest enzymatic activity (2981.74 U/mL). For industrial-scale up, a two-stage approach was followed. First, the optimisation of oxygen transfer parameters, agitation, and aeration was done in 20 L bioreactor trials. The optimised process was then scaled up to a 1000 L pilot-scale reactor, integrated with digital control systems for real-time temperature, pH, and mixing control. The enzymatic solution (0.05–3.5% v/v) was added in several discrete doses during hydrolysis of maize gluten substrates over 18–30 h. The resultant hydrolysates were formulated into a biostimulant prototype. The product obtained was compared with control formulations in field trials. This study shows effective large-scale manufacture of microbial EAP in support of a shift towards a more eco-friendly and efficient productive agri-biotech processes.

Partial grains germination and targeted lactic acid bacteria fermentation: a powerful duo to enhance nutritional and functional quality in the baking industry

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Initial studies investigating the combined use of cereal germination and targeted fermentation with lactic acid bacteria (LAB) have produced promising and convergent results, demonstrating significant improvements in the nutritional, technological, and sensory qualities of cereal, pseudocereal, and legume flours. In this study, type III sourdoughs were developed from sprouted wheat and barley flours fermented with selected LAB to evaluate their full potential. In addition to previous works, this study comprehensively compares these innovative ingredients with conventional approaches commonly employed in the baking industry. Using a predictive model based on single-strain fermentation data, two tailored ternary starter consortia were designed: *Furfurilactobacillus rossiae* (CR5), *Weissella confusa* T6B10, and *Lactiplantibacillus plantarum* SB88.B4 for sprouted wheat flour; and *Leuconostoc pseudomesenteroides* DSM 20193, *L. plantarum* 7A, and *F. rossiae* (CR5) for sprouted barley. Fermentation markedly increased peptides (450% in wheat, 520% in barley) and phenolic compounds (344% and 261%), enhancing antioxidant activity by 100% and 40%, respectively. Breads made with barley-based sourdough showed superior nutritional qualities, including higher dietary fiber, improved protein digestibility (81.14%), reduced predicted glycemic index (84.78%), and strong ACE inhibitory activity (73%). Rheological tests confirmed that doughs with these type III sourdoughs matched the performance of conventional type II sourdoughs combined with enzymatic enhancers, validating their suitability for baking. Sensory analyses highlighted the distinct appeal of each: sprouted wheat sourdough breads featured improved crust and crumb color, while barley sourdough breads stood out for bran aroma, toasted notes, and balanced acidity. This approach enabled the development of a multifunctional ingredient that reduces the need for additives and offers an economically and environmentally sustainable solution. However, future efforts must address challenges in industrial implementation, germination process scalability, bioactive compound stability after digestion, and full consumer acceptance.

Characterization of bacterial cellulose produced by *Komagataeibacter* sp. using agri-food byproducts as cost-effective substrates.

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Bacterial cellulose (BC) is a commercially valuable biopolymer renowned for its high purity, mechanical strength, and nanofibrillar structure. However, the economic feasibility of large-scale BC production is constrained by the high cost of conventional culture media. This study explores the biosynthesis and physicochemical characterization of BC produced by the previously selected *Komagataeibacter xylinus* DSM 2004 and *Komagataeibacter rhaeticus* LM4 using beet molasses (BM) and brewer's spent grains (BSG) as cost-effective growth media. Cultivation conditions were optimized through the use of sodium phosphate buffer and 0.5% (w/v) yeast extract. Subsequent, static fermentation was conducted for 10 days at 28 °C, followed by evaluation of BC yield, bacterial cell load, sugar consumption, and gluconic acid production. The obtained BC films were characterized using Fourier-transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), and mechanical testing. The results demonstrated enhanced BC production, affirming the efficacy of the prior optimized protocol. DSM 2004 demonstrated superior BC synthesis, yielding 3.11 g/L in BM and 1.55 g/L in BSG, whereas LM4 produced 1.80 g/L and 1.46 g/L, respectively. Both strains exhibited robust growth kinetics, though incomplete sugar utilization was observed. The sodium phosphate buffer effectively stabilized pH during the fermentation. However, an increase in pH was observed in BM medium inoculated with DSM 2004 due to the consumption of gluconic acid previously produced. BC thickness significantly varied (0.0100–0.0392 mm), influencing mechanical performance. DSM 2004- derived BC from BM medium demonstrated the highest tensile strength (127.55 MPa) and Young's modulus (49.91 MPa), albeit with low elongation at break (4.95 %). FTIR spectra confirmed cellulose I in all samples, while XRD indicated crystallinity differences, with the highest crystallinity index in DSM 2004- produced BC from BM. These results underscore the potential of BM and BSG as sustainable, low-cost substrates for efficient BC production, aligning with circular bioeconomy principles and waste valorisation strategies.

Paper-based packaging and microbial ecosystems: a circular approach to food safety and environmental sustainability

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The growing demand for sustainable food packaging is paralleled by the need to preserve microbial balance across the food value chain. Paper-based packaging offers a renewable, recyclable, and compostable alternative that aligns with circular economy principles and supports food safety. Smurfit Westrock is committed to developing solutions that integrate industrial innovation with environmental responsibility.

Packaging plays a crucial microbial role at all stages of the food chain—from soil microbiota in agriculture to foodborne microbes in processing, and environmental microbiota in end-of-life scenarios. Understanding these interactions opens new opportunities: packaging can be designed not to disrupt, but to support microbial ecosystems. Compliance with MOCA certifications and evolving regulatory frameworks further underscores this shift.

Smurfit Westrock's fibre-based solutions embody this microbiologically-aware approach:

- AgroPaper™: a compostable mulch paper that promotes soil respiration and microbial diversity while replacing plastic films.
- PaperNet: a recyclable net for fresh produce that maintains microbial freshness, ideal for local, plastic-free supply chains.
- Safe&Green™ Punnet: a plastic-free, 100% paper tray offering direct food contact certification, microbial safety, and optimal airflow.
- Paper-based Cans: recyclable, food-contact certified solutions for baked goods, designed to minimize microbial contamination and extend shelf life.

These innovations support the goals of the EU Packaging and Packaging Waste Regulation (PPWR) and ESG frameworks, while contributing to microbial equilibrium and waste reduction in food systems.

We invite food manufacturers, microbiologists, sustainability researchers, and packaging innovators to collaborate on scaling these ready-to-deploy solutions. Together, we can advance packaging that respects microbial life and supports a truly circular food future.

Engineered yeasts for sustainable dsRNA production: molecular tool development and fermentation optimization

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Viticulture faces major sustainability challenges due to resource-intensive practices and the large volumes of byproducts such as grape pomace. The widespread use of chemical pesticides also raises environmental and health concerns, highlighting the need for eco-friendly alternatives. This project investigates the valorization of grape pomace as a substrate for engineered yeasts producing double-stranded RNA (dsRNA), a biotechnological tool to combat grapevine diseases like downy mildew and grey mold. To address the high cost of *in vitro* dsRNA synthesis, the study focuses on optimizing *in vivo* production and delivery. Genome editing strategies are used to express dsRNA molecules targeting plant susceptibility genes, promoting sustainable crop protection.

Chardonnay pomace from the Franciacorta region was separated from stalks, thermally treated (50, 65, 75 °C), dehydrated, and milled. Microbiological analyses showed reduced contamination at 65 °C and 75 °C. Enzymatic hydrolysis with cellulase increased glucose content by ~25%. Growth trials with *Saccharomyces cerevisiae* EC1118 and *Pichia pastoris* GS115 showed superior performance of *S. cerevisiae*. A CRISPR/Cas9 strategy was used to generate an EC1118 mutant lacking the *RNT1* gene, enhancing dsRNA accumulation.

Two delivery approaches were developed targeting *VviLBDIf7*, a susceptibility gene in Vitis vinifera: (1) expression of hairpin RNA from the p426-Nat plasmid for *in vivo* self-annealing, and (2) encapsidation via MS2 bacteriophage coat proteins, using the pESC-URA plasmid co-expressing dsRNA fused to a 19-nt MS2 operator and the gene for the MS2 gene. In both cases, expression was confirmed within 72 hours, and dsRNA was purified by DNase/RNase treatment. Yield optimization and *in vivo* assays are ongoing. These efforts contribute to more sustainable viticulture, aligned with circular economy principles.

Microbial diversity and dynamics of fruit-flavored traditional yoghurt 'Ergo' in Southern Ethiopia

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This study investigated the microbial diversity, dynamics and sensory acceptance of fruit-flavored traditional Ethiopian yoghurt, 'Ergo', supplemented with fruit juices from mango (*Mangifera indica*), papaya (*Carica papaya*), and avocado (*Persea Americana*, a source of Zn). Ergo samples were collected from Hawassa and Yirgalem in Southern Ethiopia. Fruit juices (mango and papaya) were substituted into Ergo at 0%, 5%, 10%, 15%, 20% and 25% (v/v) concentrations. Sensory analysis was conducted, and the lactic acid bacteria (LAB) involved in the fermentation process were characterized using Rep-PCR and 16S rRNA gene sequencing on the 15% mango formulation (the most preferred) and the control Ergo. The results revealed that Lactobacillus and Enterococcus were the predominant genera involved in Ergo fermentation, and their diversity and distribution were influenced by location and the duration of fermentation. Of the 234 LAB isolates identified, *Limosilactobacillus fermentum* (24.36%) and *Enterococcus songbeiensis* (18.37%) were the most abundant, while *Lactiplantibacillus plantarum* was the lowest (0.85%). *Enterococcus faecalis* was exclusively isolated from Yirgalem Ergo, whereas *Lentilactobacillus parabuchneris* was found only in Hawassa Ergo samples. *Limosilactobacillus fermentum* predominated on day 4 (F4) of Ergo fermentation in Hawassa (28%), and on day 2 (F2) in Yirgalem (37.5%). The findings open perspectives on the possible use of autochthonous LAB species as starter cultures for optimizing the fermentation processes and scaling up Ergo production in Ethiopia.

Valorization of grape pomace and carob flour in plant-based gurts: starter selection, phenolic profiling and antioxidant efficacy on human keratinocytes

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Plant-based yogurt alternatives, or "gurts," are a promising option for promoting sustainable diets and food systems. Their market growth is steadily increasing, and the search for new ingredients that can supply functional compounds while providing market differentiation continues. In this study two novel rice-based gurts supplemented with carob pulp powder, the by-product of locust bean gum production from carob seeds, and grape pomace, the solid residue remaining after grapes are pressed for winemaking, were designed. Several lactic acid bacteria were screened and two *Lactiplantibacillus plantarum* strains were selected based on the acidification kinetics and ability to improve gurts radical scavenging activity. *Ex vivo* assays on human keratinocyte NCTC 2544 cells were conducted to confirm the *in vitro* activity observed. None of the gurts had cytotoxic effect, when used at different concentrations, moreover, the presence of carob- and grape pomace-gurts counteracted the negative effect of H_2O_2 used to induce oxidative stress in the cells. Although differences were not statistically significant between gurts before and after fermentation, the expression of genes (*SOD2* and *HMOX-1*), indicators of cellular oxidative stress, was significantly lower in fermented samples. To clarify the role of fermentation on gurts antioxidant potential, phenolic compounds were selectively extracted and characterized through HPLC-MS techniques. Overall, the total amount of phenolic compounds was higher in fermented gurts compared to not fermented samples. An intense metabolic activity on phenolic acids and glycosylated phenolic compounds, involving microbial decarboxylases and esterases, was observed, especially in fermented carob gurt, leading to the increase of compounds with higher bioactivity and bio-availability (e.g., pyrogallol) than its precursors. This work demonstrated that grape pomace and carob flour might be used as nutritional improvers, while fermentation with selected starters represents a sustainable and effective option for exploiting their potential and promoting their valorization.

Investigating the effect of low-power ultrasounds on the optimization of bioprocesses in a panel of evolutionary diverse microorganisms

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Bioprocesses, i.e. processes employing living cells or their components to obtain products for the food, feed and energy sector, represent one of the most promising alternatives to conventional productions. Their wide application is currently limited by low bioconversion, productivity and high extraction costs, therefore, research interest has focused on optimizing upstream and downstream phases. Ultrasounds (US) are a promising, environmentally friendly technology to optimize bioprocesses. They propagate as pressure waves in a liquid medium inducing both chemico-physical effects, improving substrate diffusion and aeration in the culture medium, as well as biological effects, mediated by mechanosensing, i.e. the ability of cells to sense mechanical cues in their microenvironment, through mechanoreceptors, conserved in walled organisms. US Effects can include increased membrane permeability, altered enzymatic activity as well as shifts in metabolic pathways. To study the effect of low-power US on bioprocesses, a Sono-(Photo)BioReactor was developed and tested on a panel of diverse microorganisms that includes two Prokaryotes and three Eukaryotes: *Escherichia coli* (Enterobacteriales) a model organism widely employed for heterologous protein expression, *Thricormus variabilis* (Nostocales) photosynthetic, nitrogen fixing filamentous cyanobacterium; *Saccharomyces cerevisiae* (Saccharomycetales), traditionally employed in the food industry, whose biomass is increasingly considered a valuable product, and two green microalgae: *Chlorella vulgaris* (Chlorellales) classified as a food and functional food ingredient and *Desmodesmus sp*. (Sphaeropleales), selected for its resistance to harsh environments. The aim of this research is to evaluate the effects of US stimulation on biomass and metabolite yields, by optical and biochemical techniques. Gathered data show US induced increased biomass production in *Chlorella vulgaris* CCAP 211/12 and *Desmodesmus sp.* VRUC 281, and a strain-specific effect on protein and carbohydrates content. Ongoing studies will focus on *E.coli* growth rate and heterologous protein expression, as well as on *S.cerevisiae* biomass production.

Antifungal potential of kiwi vinegar for clean-label applications in the baking industry

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Despite advances in food processing and packaging, fungal spoilage remains a persistent issue in the baking industry, contributing to substantial environmental and economic losses. As consumer demand shifts toward clean-label alternatives, the search for effective natural preservatives is intensifying. In this study the antifungal potential of kiwi vinegar as a natural alternative to synthetic preservatives in baked goods was investigated. Two kiwi vinegars, produced by L'Agro del Kiwi through a patented process similar to that used for wine vinegar, but starting from a must obtained from undersized kiwi fruits discarded from the commercial supply chain, and aged for 2 (AK2) and 5 (AK5) years, respectively, were evaluated. Twenty-two fungal species (commonly isolated as spoilage molds in bakery products), as well as yeasts and lactic acid bacteria typically used as starters in the baking industry, were used as indicator organisms. Compared to acetic acid, kiwi vinegars caused only a mild inhibition of *Saccharomyces cerevisiae*, *Lactiplantibacillus plantarum*, and *Fructilactobacillus sanfranciscensis*, while displaying strong antifungal activity against spoilage fungi, including *Penicillium roqueforti* and *Aspergillus niger*. Growth inhibition was attributed to a synergic effect of acetic acid and phenolic compounds, originating from the kiwis and their fermentation.

Vinegars were tested both as ingredients and sprayed in model breads. Breads made with 8.3–9.2% of AK5 and AK2, respectively, increased antioxidant activity (up to 90%), and extended shelf-life compared to control bread. Indeed, mold growth was delayed by 7 days in bread slices inoculated withs spores of *P. roqueforti* and completely suppressed for 60 days in uninoculated bread. When sprayed on bread surface, kiwi vinegars delayed visible spoilage for up to 30 days. These findings highlight kiwi vinegar's potential as a clean label antifungal agent in baked goods, with broad-spectrum efficacy and minimal impact on sensory properties.

Production of 3-Hydroxypropionic acid from seaweed waste

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Microorganisms, leveraging organic resources such as agricultural products and waste, present promising opportunities for sustainable production, provided that efficient processes are established and optimized. This study proposes a greener method for producing 3-hydroxypropionic acid (3-HP) using seaweeds.

Seaweeds can be exploited to produce various commercially valuable constituents, but none of the current applications utilize compound X, which can be present up to millimolar amounts per gram of dry weight cells. Thus, producing 3-HP from X-rich algal waste supports the transition to a circular economy, where resources are utilized more efficiently, waste is minimized, and added value is maximized. We developed a cellular tool that enables the conversion of X to 3-HP while eliminating its undesirable co-product Y from the cultivation media.

Our findings indicate that the 3-HP producing enzyme Xase, expressed in *E. coli* using the pET16 vector, yielded 24 % 3-HP relative to the initial X concentration. In contrast, Labrys with the pLMB509 vector achieved a higher yield of 80 %, directly excreting 3-HP into the medium. These results show that, once optimized, this approach could significantly enhance the sustainability of 3-HP production processes.

Solid-state fermentation to enhance the nutritional value of soybean meal: optimized protocols for sustainable feed production

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Optimizing the nutritional value of raw materials intended for the feed industry is a key priority to support more efficient and sustainable animal production. Plant-based flours, especially defatted soybean meal (a byproduct of soybean oil extraction), are common sources of protein in feed formulations. However, their use is often limited by the presence of anti-nutritional factors (phytic acid, raffinose family oligosaccharides, and trypsin inhibitors) that decrease digestibility and nutrient bioavailability. In this study, solid-state fermentation (SSF) was applied to reduce these compounds and enhance soy nutritional profile. To meet industrial processing requirements and optimize microbial growth conditions, various hydration levels and corresponding water activity values were tested. The optimization process involved the use of two filamentous fungi (*Rhizopus oligosporus* and *Aspergillus orizae*), yeasts (*Saccharomyces cerevisiae*), lactic acid bacteria (LAB), and proteolytic enzymes (Alcalase® and Neutrase®), tested individually or in combination.
Preliminary results led to the exclusion of and, due to their lower performance compared to, and of enzymes, which, although effective, are less economically viable for industrial production. The combination of with chemical acidification or co-fermentation with proved to be the most effective strategies, achieving a significant increase in free amino acids, a 50–70% reduction in raffinose family oligosaccharides, and a complete elimination of trypsin inhibitor activity. Phytic acid content also showed a reduction, more pronounced in LAB co-fermented samples. Then the shelf-life of soybean meal bioprocessed according to the optimized conditions was evaluated after drying and in samples stored without drying. Overall, the protocols optimized in this study confirmed the potential of SSF to enhance the nutritional value of soybean meal for feed applications.

Industrial production of women's health-targeted fermented milk: process optimization of lactose-free skimmed milk with *Lactobacillus crispatus* BC5

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Fermented milks are recognized as effective delivery systems for probiotics. In the framework of the FUNPRO-L project, *Lactobacillus crispatus* BC5 (selected for its anti-Candida, anti-pathogen activity, and technological traits) was used to produce a fermented milk targeting women's well-being at industrial scale (50 L). Two inoculation strategies were compared: (a) co-inoculation with an exopolysaccharide-producing starter (Lyofast ST440, Sacco) and (b) addition of BC5 (Centro Sperimentale del Latte S.r.l.) at the end of fermentation. In both cases, 8 Log CFU/mL of the probiotic were added. To meet the growing consumer demand for lactose-free products, lactase was added during fermentation. The process was conducted at 43 °C until pH 4.7 was reached. The product was then stored in sterile 1 kg containers at 4°C and 8°C. pH and microbial counts were measured at T0 and after 1, 7, 14, 21, and 28 days. Samples from days 1, 14, and 21 were also analyzed for water-holding capacity, antimicrobial activity, and volatilome profiles. Probiotic viability remained stable when *L. crispatus* BC5 was added at the end of fermentation, while co-inoculation led to a 1 Log CFU/mL reduction over time. Antimicrobial activity against *Escherichia coli* and *Gardnerella vaginalis* persisted in all samples. On day 21, both formulations were nutritionally similar, except for total sugars, lower (4.5 vs. 5.6 g/100g) in the late-inoculated samples, likely contributing to their lower pH. Volatilome analysis showed that co-inoculation increased acetic acid levels (up to 10 ppm eq.), while sequential inoculation enhanced acetoin content, improving creamy and buttery flavor notes. Overall, late addition of *L. crispatus* BC5 ensured high probiotic viability, desirable sensory characteristics, and maintained antimicrobial function throughout storage. A clinical trial will assess the product's benefits for women's.

Bacterial cellulose from *Komagataeibacter* spp. strains: variability, adaptability and biotechnological potential within UMCC

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At Unimore Microbial Culture Collection (UMCC) of the University of Modena and Reggio Emilia, the preservation of microbial diversity and the advancement of sustainable biotechnological innovations are core objectives. UMCC preserves over 3000 strains of bacteria and yeasts isolated from fermented food sources. Acetic acid bacteria (AAB) represent a key microbial group, particularly *Komagataeibacter* sp., known for their ability to synthesize bacterial cellulose (BC), an exopolysaccharide with unique properties. Recent research at UMCC demonstrated the capacity of *Komagataeibacter* strains to synthesize BC from various synthetic and alternative carbon sources. The use of cheese whey (CW) and olive mill wastewater to produce BC confirmed the adaptability of AAB strains to complex substrates. Particularly, in CW many strains exceeded a productivity of 0.6 g/L/day. AAB strains also showed a high variability in BC production related to differences in cellulose synthase complex or isolation sources. However, these properties are crucial for designing sustainable production processes and support circular economy principles. Recent investigations explored the integration of AAB within complex systems, like synthetic microbial communities, where co- cultivation with other microorganisms enhances BC productivity (+50% in most of the combinations) adding functional properties. These strategies lower environmental impact while producing functionalized BC broadening BC-based material applications. These advances demonstrate the potential of UMCC strains for creating next-generation biomaterials for food, biomedical, and environmental applications.

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Development of biotechnological processes for bioplastics production from dairy waste using selected microrganisms

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The increasing demand for sustainable materials has driven interest in bioplastics produced by microorganisms using industrial byproducts as substrates. Polyhydroxyalkanoates (PHA), produced from different carbon sources and stored in microbial cells in the form of granules as energy reserves, are currently the most studied biopolymers. PHA synthesis occurs, during the fermentation process, in deficiency of essential nutrients (N, P, S) and excess of a carbon source.

This study investigated the potential of *Azohydromonas lata* DSM 1123 for the biosynthesis of polyhydroxyalkanoates using scotta (ricotta cheese exhausted whey), a lactose-rich dairy by-product, as a carbon source. Before the cultivation process, scotta was subjected to several pre-treatments to modulate nitrogen content. The fermentation process was optimized to enhance PHA yield, assessing microbial growth, and evaluating polymer accumulation. Fermentation conditions (culture medium for the microorganism propagation, inoculum size, time, and temperature of incubation) were selected to maximize polymer synthesis, in flasks experiments. The PHA production was then scaled up, under stable and monitored pH, temperature, and stirring conditions, using a bioreactor. Results demonstrate that scotta is overall a suitable substrate for PHA production. The maximum amount of polymer recovered (with a 32.7% yield PHA/dry biomass weight), was achieved using a substrate consisting of 75% pasteurized and 25% sterilized scotta. Gas chromatography, gel permeation chromatography and differential scanning calorimetry analyses confirmed high purity polyhydroxybutyrate as the polymer synthesized. Overall, the interest in optimizing this production system contributes to the valorization of dairy industry waste, promoting circular economy principles while offering an eco-friendly solution for bioplastic manufacturing.

Exploring the role of *Lactiplantibacillus plantarum* and its exosomes in skin health: wound healing, antioxidant, and anti- inflammatory effects

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The human skin microbiota is recognized as a key factor in maintaining epidermal homeostasis and promoting skin health. In this study, skin-associated bacterial strains were isolated and their potential interactions with human skin cells were investigated. Among the isolates, *Lactiplantibacillus plantarum*, known for its probiotic profile and safety, was selected for in-depth characterization.

Promising effects on wound healing and skin hydration were observed *in vitro*, as indicated by enhanced re-epithelialization and increased filaggrin expression in keratinocytes. These outcomes led to the hypothesis that bacterial extracellular vesicles might mediate the observed biological effects. Consequently, exosomes produced by *L. plantarum* were isolated and analyzed, and were found to elicit stronger responses than the intact bacterium.

Further analyses demonstrated that these exosomes possess significant antioxidant and anti-inflammatory activities, including a marked reduction in inflammatory responses triggered by *Cutibacterium acnes*. To gain insight into the underlying mechanisms, the small RNA cargo of the exosomes was characterized and their transfer into keratinocytes was confirmed. Post-transcriptional regulation of gene expression was observed, resulting in the downregulation of specific inflammatory targets.

These findings reveal a novel mechanism by which *L. plantarum* may contribute to skin health and support the potential application of bacterial exosomes as next-generation postbiotics in dermatological settings.

Application of whey protein-based anti-*Listeria* biopackaging solutions to preserve tradition and ensure safety of Canestrato Tradizionale Siciliano cheese

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Canestrato Siciliano (CS) cheese is a traditional fresh pressed cheese made from raw cow's milk, distinguished by its characteristic cylindrical shape, uniform structure, and ivory-white color. The use of raw milk and the short ripening period of 20 days pose a risk of potential dissemination of *Listeria monocytogenes*. In detail, twelve samples of CS were obtained from six different local producers in southeastern Sicily. Cheese samples were analyzed to assess their microbiological, chemical, and compositional profiles. Overall, results revealed considerable variability among samples within the fourteen microbial groups detected, highlighting the influence of local environmental and processing conditions on microbial communities. All samples exhibited high microbial loads (~10^8 CFU/g) for total mesophilic bacteria, mesophilic rods and cocci, and thermophilic lactic acid bacteria. Statistically significant differences ($p < 0.05$) were observed for mesophilic and thermophilic *Lactococcus* spp. and mesophilic *Lactobacillus* spp. Chemical analyses revealed considerable variability in total solids content ($p < 0.05$), further reflecting the diversity among production practices. The metagenomic analyses and the profiling of volatile organic compounds (VOCs) have been applied to achieve a deeper understanding of the interaction between microbial populations and flavor development. In order to ensure safety and support environmental sustainability of CS cheese, an innovative whey protein-based packaging solution activated with a commercial anti-*Listeria* phage (Phageguard Listex, Phageguard / Micreos Food Safety, Wageningen, Netherlands) has been considered as a strategy to reduce the non-biodegradable materials in the packaging. The in *vitro* analysis on phage solution and packaging systems are in progress in order to evaluate their efficiency.

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Catalyse platform: building a community of practice to foster innovative solutions for food safety actors

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Food safety is a critical aspect throughout the entire food chain, influencing public health, economic stability, and consumer trust. In recent years, extensive research and numerous innovative solutions have been developed to address this challenge. However, several barriers impede effective knowledge sharing among food system actors—researchers, industry representatives, innovators, and policymakers—and limit the adoption of these advances, hindering collaboration and slowing progress in food safety. The CATALYSE project aims to bridge this gap between food system actors by developing a Community of Practice (CoP) platform. This approach will establish a robust, sustainable, and inclusive community—both online and in practice—that addresses the needs of all stakeholders, from farm to fork, to ensure food safety. The CATALYSE CoP is a shared space where all the actors in the food chain can meet and collectively define their needs and the opportunities that innovative solutions can offer. By linking these actors, the aim is to foster food safety innovation, particularly for industry stakeholders, providing win-win solutions. A Stakeholder Engagement Strategy was employed to identify the main needs and priorities related to food safety from different stakeholders. Thematic Chapters were defined considering the priority areas to facilitate the transfer of knowledge and innovative solutions related to food safety. Additionally, to enhance connections between different stakeholders, the most promising startups, spinoffs, and innovative solutions were mapped, improving collaborations within the CATALYSE CoP. Furthermore, the platform offers a scientific database of reliable knowledge and educational materials -including training and courses - to increase communication in food safety advancements and improving discussions and networking between food safety actors. The CATALYSE CoP platform provides stakeholders from across the food chain with access to valuable and innovative food safety solutions, enabling rapid and reliable implementation of them throughout the entire food chain.

Innovation in pre- and post-harvest biocontrol: novel strategies and first scale-up trials for grape preservation against *Botrytis cinerea*

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Driven by the demand for sustainable agriculture, biocontrol is emerging as a crucial alternative to chemical fungicides for crop protection. This strategy focuses on the use of living microorganisms, their metabolites or other natural compounds to counteract plant diseases and preserve food quality. A critical issue in the wine industry is *Botrytis cinerea* infection, which compromises grape quality. Current management relies on chemical fungicides, but their use is restricted in pre-harvest to avoid residues in the final product, pushing towards natural solutions such as biocontrol. However, the number of microorganisms registered as active substances in the European Union remains limited, highlighting the need for improved selection and application processes. In this context, the aim of this work is to assess the performance of new microbial isolates (grapevine wood endophytes) as bioprotective microorganisms, compared to commercial agents already in use, such as *Metschnikowia fructicola* (currently reclassified as *M. pulcherrima*) and *Aureobasidium pullulans*. The study firstly focuses on post-harvest application, especially the storage phase, which represents a critical moment for the protection of fruit quality. The bio-protective potential of bacterial isolates, primarily Actinobacteria previously selected through *in vitro* assays, was evaluated on grape bunches. Laboratory-scale trials with controlled *B. cinerea* infections allowed the evaluation of microbial colonization, viability during storage, and effectiveness. Monitoring of disease incidence and severity revealed a significant reduction in fungal growth. The tested isolates showed biocontrol efficacy comparable to reference microorganisms. Further trials under industrial conditions (withering warehouses) were conducted to determine the most suitable application conditions to reduce the development of naturally present *B. cinerea*. Experiments at two different wineries tested microorganisms alone and in microbial consortia, also evaluating the role of adhesives in treatment persistence. The results, particularly encouraging under high *B. cinerea* pressure, indicate promising potential as an alternative to chemical control.

Revitalising brewers' spent grains and enriching with biogenic compounds through the fermentation of fructophilic lactic acid bacteria and yeasts

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The large output of spent grains from the brewing industry presents environmental concerns but also offers promising nutritional and functional potential for valorization by researchers and industrial stakeholders. In this perspective, we investigated how non-conventional starters like *Fructobacillus fructosus* PL22 and *Wickerhamomyces anomalus* GY1 can drive the fermentation of brewer's spent grain (BSG), a solid by-product of the brewing industry, to enrich its portfolio of bioactive compounds. While sugar reduction was comparable between started- and unstarted-BSG, the effect of the fermentation became evident through the release of key microbial metabolites (lactic and acetic acids and ethanol). Both starters generated the highest number of unique peptides, with only one previously identified as antioxidant peptide found in BSG fermented with *F. fructosus.* During fermentation, most amino acids and phenolic compounds decreased, while BSG fermented with *W. anomalus* distinctly enhanced the release of Ala, Cys and GABA, and health-promoting phenolic compounds, such as gallic acid, gallocatechin, quercetin, naringenin, kaempferol, and isorhamnetin. These metabolic changes were associated with the enhanced antifungal and antioxidant properties, which in turn positively reflected on skin protection as shown by the increased proliferation of human keratinocytes, over-expression of the filaggrin (FLG) gene, and wound healing. The power of fermentation to revitalise BSG, giving it a second life chance through the improvement of its nutritional value and further multifunctionality, was demonstrated.

Lacticaseibacillus rhamnosus for biogenic amines reduction in cheese: from gene detection to functional validation

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Biogenic amines (BAs) can accumulate in fermented foods, posing potential toxicological risks. Certain lactic acid bacteria may help mitigate BAs through enzymatic oxidation. In this study, ten *Lacticaseibacillus rhamnosus* strains were isolated from Parmigiano Reggiano cheese and screened for genes involved in tyramine and histamine production. To evaluate their potential for BAs detoxification, whole genome analysis was performed to identify the presence of oxidase-encoding genes. Two oxidative enzymes - multicopper oxidase (MCO) and FAD-dependent amine oxidase (FADOX) - were identified, and specific primers were designed for their detection. Gene presence and expression were evaluated using conventional PCR and quantitative PCR (qPCR). BAs degradation was assessed by HPLC, and the most promising strain was employed as a starter culture for Caciotta cheese production. RAPD-PCR with primer M13 revealed 4 distinct biotypes. All strains were negative for histidine and tyrosine decarboxylase genes, while both oxidase genes were detected in all strains. The MCO gene expression varied significantly among strains, whereas FADOX gene showed lower fold changes than MCO gene. HPLC analysis revealed that spermidine was the most degraded biogenic amine (BA) across all strains, with each strain degrading more than 50% of histamine and tyramine. Strain RBV03 showed the highest degradation capacity and was selected for Caciotta cheese production. Its inoculation led to a 65% reduction in total BAs content compared to the cheese control produced with a commercial starter. These findings support the application of *L. rhamnosus* as a promising adjunct or protective culture in BA-prone fermented foods, providing a microbiological strategy to enhance product safety while preserving traditional processing methods.

Different fermentation approaches to valorise the Pâté Olive Cake (POC)

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The olive oil industry generates large quantities of agro-industrial by-products, among which pâté olive cake (POC) appears fascinating for its high content of bioactive phenolic compounds, such as hydroxytyrosol and tyrosol. In this study, POC was diluted with olive mill wastewater (OMWW) and distilled water (3:2 ratio; 530 mL OMWW + 190 mL H_2O per kg POC) and supplemented with 2% glucose. Fermentations were carried out in duplicate for 14 days at 25 ± 2 °C under constant stirring, using selected lactic acid bacteria (LAB) and yeast strains isolated from fermented food. Six fermentation trials were set up: (i) uninoculated control; (ii) *Lactiplantibacillus plantarum* C11C8; (iii) *Lactiplantibacillus pentosus* L7; (iv) *Wickerhamomyces anomalus* F5.60.05; (v) *Wickerhamomyces anomalus* F5.60.01; (vi) sequential inoculation of yeast (F5.60.01+F5.60.05), followed after 72 hours by LAB blend (C11C8+L7). Strains were inoculated at 10 CFU/mL (LAB) and 10 CFU/mL (yeasts). Samples were analysed for physico-chemical and microbiological parameters. Microbiological tests confirmed absence of harmful bacteria at T14. All inoculated samples showed a steady acidification trend (reaching pH 3.76–3.97) and an overall increase in total phenolic content (TPC). Colour changes were more pronounced in LAB samples. Total soluble solids decreased progressively, reflecting microbial metabolic activity. In inoculated samples, the growth of spoilage and pathogenic microorganisms was reduced. The UHPLC/DAD analysis was applied to detect the phenolic content. Fermented samples showed higher antioxidant activity than the control (FRAP, DPPH, ABTS). Results revealed that *Lpb. pentosus* showed the most efficacy in enhancing TPC, microbiological safety, and stability of POC samples, supporting sustainability in the olive oil processing industry.

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Effects of vineyard irrigation regimes and yeast inoculation strategies on fermentation dynamics and wine sensory profile

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Alcoholic fermentation of wine is influenced by vineyard management, nutrient availability, and inoculation strategy, all of which shape microbial succession and the wine's sensory and analytical traits. This study investigated the effects of conventional vineyard management (NIT) versus late irrigation (IT), combined with either a commercial Saccharomyces cerevisiae starter (SA) or spontaneous fermentation (SP), on sugar consumption, yeast dynamics, volatile composition, and wine characteristics. SA fermentations showed consistent, linear sugar consumption, while SP fermentations were more variable due to indigenous yeast competition, confirmed by next-generation sequencing (NGS). IT musts started with higher yeast loads (7.5–7.9 log CFU/mL) than NIT (7.2–7.3), yet NIT fermentations exhibited faster early sugar consumption and yeast growth, likely driven by better nutrient bioavailability. Volatile analysis (SPME-GC/MS) showed 8–10% more volatiles in IT wines, especially alcohols (167–255 ppm eq.) and esters (218–289 ppm eq.). SP-IT samples were rich in ethyl acetate and phenylethanol, while SA wines had more fatty-acid esters and acetic acid. SA-IT wines had higher total acidity, alcohol, and glycerol than SA-NIT, which showed higher pH. SP-NIT wines displayed deeper color and more phenolics, while SP-IT wines were more acidic and astringent. Sensory analysis confirmed these differences: SA-IT wines had richer color, fuller body, and greater complexity; SA-NIT wines were more aromatic with softer tannins. SP-NIT wines achieved balanced aromatics, whereas SP-IT wines were brighter and firmer. In summary, NIT favored aromatic expression and smoother mouthfeel, while IT enhanced structure, acidity, and persistence. In conclusion, vineyard water management and fermentation protocol jointly modulate not only the rate and efficiency of sugar metabolism but also yeast ecology, aromatic development, and the ultimate sensory character of the wine.

Valorization of volatile fatty acids-enriched cheese whey by *Yarrowia lipolytica* for single cell oil production

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Food waste and by-products are often valorized through methane production; however, volatile fatty acids (VFAs) from acidogenic digestion can serve as renewable carbon sources for single cell oil (SCO) production. *Yarrowia lipolytica*, an oleaginous yeast with Qualified Presumption of Safety (QPS) status, can accumulate SCOs up to 20% of cell dry weight (CDW) on sugars and even more on hydrophobic carbon sources. This study, performed within the European project ONE EARTH, investigates SCO production by *Y. lipolytica* grown on cheese whey, a common dairy by-product, enriched in VFAs via acetogenic fermentation. Ten wild-type strains were screened on minimal medium with 0.2% acetic acid; seven showed good performance. Their growth was monitored for 72 hours across acetic acid concentrations from 0.2% to 1.0%. Strain-specific tolerance was observed. Based on their performance, three strains (Y3, RO3., RO2) were selected and tested on a VFA-enriched medium at 20°C and 5°C for 13 and 20 days, respectively. While growth was slower at 5°C, biomass was higher (Y3: 817.9 mg/100 mL), whereas lipid accumulation increased at 20°C (Y3: 79.8 mg/100 mL, 10% CDW). Fatty acid analysis showed C18:1, C18:2, and C18:3 accounted for 38–46% of total lipids. RO3. had the highest unsaturation index; Y3 yielded the most long- chain unsaturated fatty acids. Y3 was then grown on VFAs, obtained from squacquerone whey, and incubated at 20°C under static and dynamic conditions. Aeration did not enhance fat content (max 13% CDW), while static conditions increased SCO content (mainly composed of C18:1, C18:2, and C18:3, >50%) to 42% after 13 days incubation. These results support *Y. lipolytica* as a promising biocatalyst to convert dairy waste into valuable lipids for circular economy applications.

Design and characterization of low-glycemic-index snack with the inclusion of bioprocessed brewers' spent grain

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Brewers' spent grain (BSG) is a brewing by-product rich in fiber and protein, yet still underexploited in human nutrition. Given its nutritional potential, different biotechnological strategies—such as enzymatic hydrolysis and fermentation by lactic acid bacteria—have been proposed to improve its functional quality and facilitate its incorporation into food matrices. In this work, BSG underwent a combined enzymatic treatment with a commercial xylanase and fermentation with *Lactiplantibacillus plantarum* PU1, previously selected among 33 LAB strains for its metabolic and technological performance. The bioprocess led to marked acidification, high LAB growth, and an increase in antioxidant capacity.

The fermented BSG was used as an ingredient in both the dough and the filling of a novel filled snack. A full physicochemical, nutritional, and microbiological characterization of the product was carried out, including *in vitro* digestibility and glycemic index prediction, as well as an *in vivo* GI determination on healthy volunteers. The snack exhibited a high dietary fiber content (18.2 g/100 g), considerable protein levels (14.5 g/100 g), and significant amounts of bioavailable minerals, meeting EU criteria for several nutritional claims. Texture analysis revealed mechanical properties comparable to commercial products, and the snack received positive sensory evaluations from both trained panels and over 130 consumers (overall liking: 7.3/9). Microbiological stability and oxidative status were maintained over 8 months of shelf life. The product exhibited a low predicted glycemic index (pGI: 58.8), which was confirmed by an *in vivo* GI value of 48.5, allowing it to be classified as a low-GI food. These results demonstrate how targeted biotechnological processing can effectively valorize agro-industrial by-products like BSG, enabling the development of nutritionally balanced, shelf-stable, and consumer- accepted snack foods with reduced glycemic impact and improved sustainability.

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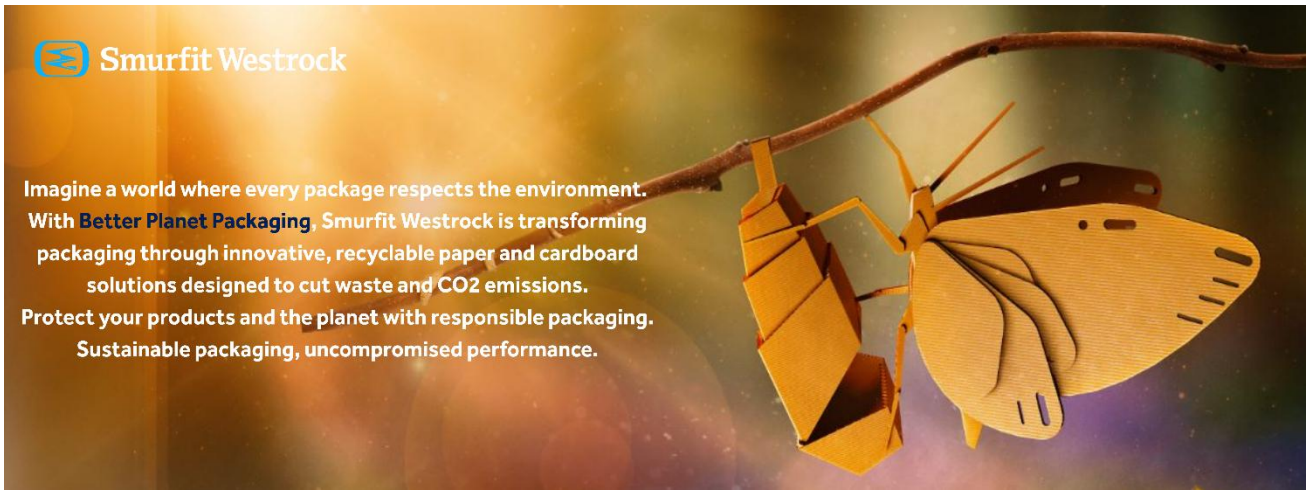
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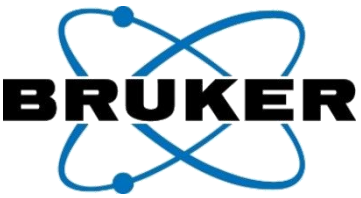


















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