ECCO XLII Meeting

"Microbe & Microbiome Management for a Better Planet"

ECCO | European Culture Collections' Organisation

Book of Abstracts

Editors: Antonio Moretti, Giancarlo Perrone

ISM

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ITEM Agri-Food Microbial Culture Collection

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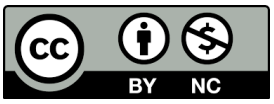

Dear Colleagues,

we are very pleased to announce that the XLII European Culture Collections' Organisation (ECCO) meeting will be held in the amazing city of Bari (Apulia region, Southern Italy), from 18 to 20 September 2024.

The ECCO meeting is organised by the Institute of Sciences of Food Production of the National Research Council of Italy (CNR-ISPA), and sponsored by the CNR-ISPA Agri-food Microbial Culture Collection (ITEM), and the International Society on Mycotoxicology.

The meeting will be focused on the study, management and valorisation of Microbe & Microbiome, as biological resources and bio-systems crucial tools for a better understanding of biological systems, to support quality of life and promote the transition towards a better planet. The conference promotes a holistic and integrated approach on the value of microbial resources and Microbial Biological Research Centers (mBRCs): i) to support basic and applied transdisciplinary sciences, ii) as resilience factors to deal with global changes and sustainable development, and iii) to promote human and animal health in the framework of a OneHealth perspective.

ECCO supports cooperation and scientific exchanges concerning all the activities of mBRCs. Annual ECCO meetings represent an opportunity for the scientific community of the field, to discuss current trends and future development in the culture collections activities.

We would like to invite you to be part of the XLII ECCO meeting that will take place at "Sala Aldo Moro" (Dipartimento di Giurisprudenza), University of Bari "Aldo Moro", near the central train station and the wonderful historic city centre.

Director of CNR-ISPA

Antonio Moretti

Curator of ITEM Collection

Giancarlo Perrone

Wednesday 18

1.00 p.m. - 2.30 p.m.	Registration and Poster placing
2.30 p.m. - 3.00 p.m.	**Welcome addresses:** Rector of University of Bari - *Prof. Stefano Bronzini*; President of CNR Research Area - *Cinzia Giannini*; Major of Bari - *Vito Leccese*; President of the European Culture Collections' Organisation (ECCO) - *Dr Gerard J. M. Verkley;* Co-Chairs of XLII ECCO Meeting - *Antonio Moretti* and *Giancarlo Perrone*
3.00 p.m. - 4.30 p.m.	**Session 5 – Microbiomes preservation and exploitation** - Chairs: Ferrara Massimo (Italy), Aznar Rosa (Spain)
3.00 p.m. - 3.25 p.m.	Microbiomes for the Industry - *Porcar Manuel, Spain* (Invited Speaker)
3.25 p.m. - 3.40 p.m.	Exploring soil microbiome preservation strategies: culturable fraction, metabolic profiling and metagenomics - *Visca Andrea, Italy*
3.40 p.m. - 3.55 p.m.	Microbiome Biobanking: The missing link - *Kostic Tanja, Austria*
3.55 p.m. - 4.10 p.m.	Cryopreservation and recovery of a complex hypersaline microbial mat community - *Grego Michele, France*
4.10 p.m. - 4.25 p.m.	Direct injection Mass Spectrometry for the Real-Time volatilomics in Food System Microbiomes: the potential of providing temporal dimension in multi-omics studies - *Corvino Antonia, Italy* Closing remark of session (5 min)
4.30 p.m. - 5.00 p.m.	**Coffee break + poster session**
5.00 p.m.- 6.30 p.m.	**Session 2– Microbes from farm to fork** - Chairs: De Vero Luciana (Italy), Oivanen Pekka (Finland)
5.00 p.m.- 5.25 p.m.	Using microbial diversity or complex communities for valorization of side-streams for food applications -*Bang-Berthelsen Claus Heiner, Denmark* (Invited Speaker)
5.25 p.m. - 5.40 p.m.	Are isolation, identification and preservation of microbial strains still useful for food applications? - *Baruzzi Federico, Italy*
5.40 p.m. - 5.55 p.m.	Soil Health and Agri-Food System Sustainability from a microbiology perspective: A Data-Driven Approach for Agricultural Policy and Practices - *Bevivino Annamaria, Italy*
5.55 p.m. - 6.10 p.m.	Be a QPS or not to be, that is the Quirky Paradox of Safety - *Chessa Luigi, Italy*
6.10 p.m. - 6.25 p.m.	Investigation on the microbial evolution of cow milk in the passage from stable to mountain pasture, and evaluation of Bitto cheese microbial community - *Zago Miriam, Italy* Closing remark of session (5 min)
7.00 p.m.	**Tour + Welcome Party**

Thursday 19

9.00 a.m. - 10.30 a.m.	Session 3 – Pathogenic and beneficial aspects of microbes in human and animal health - Chairs: Hurtado Ortiz Raquel (France), Susca Antonia (Italy)
9.00 a.m. - 9.25 a.m.	Studying host-microbe interactions in health and disease using germ-free and gnotobiotic mouse technology - *Vereecke Lars, Belgium* (Invited Speaker)
9.25 a.m. - 9.40 a.m.	Using DNA Metabarcoding as a non-invasive tool for the conservation of the critically endangering Seychelles bat *Coleura seychellensis* - *Mattarelli Paola, Italy*
9.40 a.m. - 9.55 a.m.	Riboflavin overproducing food-grade bacteria: genomic basis, biotechnological applications and perspectives - *Capozzi Vittorio, Italy*
9.55 a.m. - 10.10 a.m.	Defining the *Enterobacter cloacae* species complex, with particular emphasis on *Enterobacter hormaechei* - *Rahi Praveen, France* Closing remark of session (5 min)
10.15 a.m. - 10.30 a.m.	Sponsor Session
10.30 a.m. - 11.00 a.m.	**Coffee break + poster session**
11.00 a.m. - 12.30 p.m.	**Session 4 – Microbial life in extreme habitats: a 21st century challenge** - Chairs: Varese Giovanna Cristina (Italy), Sedláček Ivo (Czech Republic)
11.00 a.m. - 11.25 a.m.	Diversity of microalgae in low pH environments: fifty years of the ACUF Collection, from strain to microbiome conservation and exploitation - *Pollio Antonino, Italy* (Invited Speaker)
11.25 a.m. - 11.40 a.m.	Life at the limits: diversity, adaptation strategies and bioprospecting of microbes living in Arctic deep-sea habitats (INDEPTH) - *Kaczorowska Anna-Karina, Poland*
11.40 a.m. - 11.55 a.m.	Cold-adapted carboxylic ester hydrolases from two Antarctic *Psychrobacter* strains: genomic analyses and in-vitro studies - *Cattaneo Andrea, Italy*
11.55 a.m. - 12.10 p.m.	Cold-adapted yeasts: a restricted club of extremophilic organisms - *Buzzini Pietro, Italy*
12.10 p.m. - 12.25 p.m.	Preliminary investigations of alkali-tolerant fungi in stromatolites from Lake Salda (Burdur province, SW Türkiye) - *Cecchi Grazia, Italy* Closing remark of session (5 min)

12.30 p.m. - 2.00 p.m.	**Lunch**
2.00 p.m. - 3.30 p.m.	**Session 1 – Microbes for environmental sustainability, under a climate change scenario** - Chairs: Turchetti Benedetta (Italy), Kermode Anthony (United Kingdom)
2.00 p.m. - 2.25 p.m.	The importance of fungi for food security and One Health under a climate change scenario - *Lange Lene, Denmark* (Invited Speaker)
2.25 p.m. - 2.40 p.m.	Fungi in marine plastisphere: ecological role and biotechnological potential - *Varese Giovanna Cristina, Italy*
2.40 p.m. - 2.55 p.m.	Exploiting the agri-food waste and by-products potential for bioplastic production through Haloferax mediterranei fermentation - *Montemurro Marco, Italy*
2.55 p.m. - 2.10 p.m.	Plant growth-promoting bacterial consortia isolated from halophytes to improve crop response to salinisation and climate change - *Racioppo Angela, Italy*
2.10 p.m. - 2.25 p.m.	Genomic and metabolomic characterization of a new toxic cyanobacteria - *Rita Cordeiro, Portugal* Closing remark of session (5 min)
3.30 p.m. - 4.00 p.m.	**Coffee break + poster session**
4.00 p.m. - 5.30 p.m.	**Session 6 – Microbes and Citizen Sciences: through the lens of public opinion** - Chairs: Moretti Antonio, (Italy) Perrone Giancarlo (Italy)
4.00 p.m. - 4.25 p.m.	The Isala project: characterizing the female microbiome through citizen science - *Wittouck Stijn, Belgium* (Invited Speaker)
4.25 p.m. - 5.25 p.m.	Side show for citizen - interactive demonstrations
5.25 p.m. - 5.45 p.m.	Results of the Survey on microbial biodiversity and taxonomy - *Felis Giovanna, Italy*
5.45 p.m. - 7.30 p.m.	**ECCO General Assembly**
8.30 p.m.	**Social Dinner**
Friday 20	
9.30 a.m. - 11.45 a.m.	**Session 7 – Advanced approaches in taxonomy, phylogeny and functional genomic** - Clermont Dominique (France), Masiello Mario (Italy)
9.30 a.m. - 9.55 a.m.	Metabarcoding with Nanopore MinION: not only 16S or ITS - *Faino Luigi, Italy* (Invited Speaker)
9.55 a.m. - 10.10 a.m.	Genomic overview over 100 cyanobacterial strains: taxonomy, microbiome and biosynthetic gene clusters - *Rúben Luz, Portugal*
10.10 a.m. - 10.25 a.m.	Drivers of aquatic fungal diversity and ecological functions throughout Europe: Biodiversa+ FUNACTION and MoSTFun projects - *Marchese Pietro, Italy*
10.25 a.m. - 10.40 a.m.	ITSoneDB v1.144 and BioMaS@ITSoneWB: two ELIXIR-IT main resources for amplicon based mycobiome investigation - *Defazio Giuseppe, Italy*
10.40 a.m. - 11.10 a.m.	**Coffee break + poster session**
11.10 a.m. - 11.25 a.m.	The new challenge in virus taxonomy: a binomial nomenclature for virus species - *Rubino Luisa, Italy*
11.25 a.m. - 11.40 a.m.	Genomic insights into Mrakia: expanding horizons in microbial biotechnology - *Di Cesare Francesca, Italy* Closing remark of session (5 min)
11.45 a.m. - 1.20 p.m.	**Session 8 – Networking, services, quality and data management of Microbial culture collections** - Chairs: Lima Nelson (Portugal), Verkley Gerard (Netherlands)
11.45 a.m. - 12.10 p.m.	MIRRI-ERIC as a microbial, genetics and data resources hub to advance scientific discovery - *Portugal Melo Ana, Portugal* (Invited Speaker)
12.10 p.m. - 12.25 p.m.	PLAVIT, the Italian Plant Virus Collection in 2024 - *Accotto Gian Paolo, Italy*
12.25 p.m. - 12.40 p.m.	How the MIRRI-PT has been developed in its smart specialisation and cutting-edge technologies to offer better services - *Lima Nelson, Portugal*
12.40 p.m. - 12.55 p.m.	The Italian network of microbial culture collections: an overview on management and sustainability of the future research infrastructure MIRRI-IT – *Moretti Marino, Italy*
12.55 p.m. - 1.10 p.m.	Managing change within a culture collection: A case study on the challenges within quality, data management and infrastructure within CABI's collection - *Kermode Anthony, United Kingdom* Closing remark of session and Ecco meeting (15-20 min)
1.30 p.m. - 3.00 p.m.	**Closing lunch**

Abstracts Summary

Session 1 – Microbes for environmental sustainability, under a climate change scenario

Thursday 19th September, 2:00pm

Chairs: Turchetti Benedetta (Italy), Kermode Anthony (United Kingdom).

The importance of fungi for food security under climate change scenario

Lange, Lene[1]

[1] *LL-BioEconomy, Denmark*

Fungi can play a major role in improving food security, achieved by upgrading what is now wasted.This is of special importance, when climate change is causing serious obstacles for agricultural food production. Fungal enzymes can valorize crop residues, agro-industrial side-streams and left over food, all being food compatible resources, now underutilized or even wasted. Enzymes can open recalcitrant structures and enable the recovery of nutritious components such as proteins, dietary fibers, vitamins, lipids and antioxidants from such bioresources. The fungi instrumental for such significant improved use of the (global and local) bioresources are well studied; making it obvious that also a rather low number of fungal strains, can be key for sustainable and safe improved use of the bioresources. Significant progress could be made if many more throughout the world had access to such strains and how to use them. Culture collections can play a vital and major role in making such an ambitious plan into reality. Proposal: Each culture collection establishes and promotes a set of cultures of fungi (and bacteria) which are documented to be safeand shown to be efficient and with no strings attached, with regard to use. Such set of cultures, being fungal strains, producing blends of biomass degrading enzymes; or fungal strains, easy to use for recombinant production; or fungal hosts, for biological production (preferably efficient also when grown on residues); or fungi for fermentation of residues, resulting in tasty, nutritious and gut health promoting food. The Culture collections are hereby encouraged to organize collaborative projects and seek funding (public or philanthropy) for developing such sets of cultures for Circularand Biobased food production, including funding for providing such sets of cultures in countries or regions, where climate change is seriously challenging food security, nutrition and health.

Fungi in marine plastisphere: ecological role and biotechnological potential

Spina, Federica[1]; Stilo, Giulia[1]; Venice, Francesco[1]; Fiorin, Anita[1]; Reginato, Alessia[1]; Florio Furno, Matteo[1]; Bracco, Perangiola[1]; Dodhia, Maya[2]; Zanetti, Marco[3]; Posth, Nicole R.[2]; Varese, Giovanna Cristina[1]

[1] University of Torino, Department of Life Sciences and Systems Biology, Torino, Italy; [2] University of Copenhagen, Department of Geosciences and Natural Resource Management Geology, Sedimentary Systems, København, Denmark; [3] University of Torino, Department of Chemistry, Torino, Italy

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Plastic released into the environment represents one of the most important environmental problems with important repercussions on the functionality of ecosystems and on the health of animals, including humans. Every year, marine environment receives more than 10 million tons of plasticwhich is quickly colonized by microorganisms. The co-occurrence of bacteria, fungi and microalgae creates a dynamic scenario which remains largely unexplored as regard the fate of the different plastics (traditional versus biodegradable plastics), the effect of abiotic features as salinity and themicrobes involved in the possible biodegradation.
In the present study, the marine plastisphere of two Danish locations (characterized by different salinities) was studied through culture dependent and independent techniques, unveiling a heterogeneous mycobiota.
The fungal community was various, with a strong role of the site, the plastic polymer (biodegradable polymer versus a non-biodegradable polymer) and environmental parameters as salinity. Hundreds of filamentous fungi and yeasts were isolated, mostly associated with Ascomycota phylum. Unveiling the hidden biotechnological potential of marine fungi is of major interest, focusing ontheir capability to transform plastic polymers. Some fungi were able to use different plastics as sole carbon source and capable of degrading biopolymers. The extent and the kinetics of such processare currently under investigation via respirometry analysis. Moreover, the genome annotation of some of these strains highlights the presence of putative Plastic Active Enzymes (PAZy).

Acknowledgements
Part of this work was granted by the European Commission—NextGenerationEU, Project "Strength-ening the MIRRI Italian Research Infrastructure for Sustainable Bioscience and Bioeconomy", coden. IR0000005.

Exploiting the agri-food waste and by-products potential for bioplastic production through *Haloferax mediterranei* fermentation

Montemurro, Marco[1]; Longo, Angela[2]; Fanelli, Francesca[1]; Villano, Marianna[3]; Rizzello, Carlo Giuseppe[2]

[1] *Institute of Sciences of Food Production, National Research Council (ISPA-CNR), Bari, Italy;* [2] *Department of Environmental Biology, "Sapienza" University of Rome, Italy;* [3] *Department of Chemistry, Sapienza University of Rome, Rome, Italy*

Several microorganisms can synthesize polyhydroxyalkanoates (PHA) which represent environmentally friendly substitutes for conventional plastic. The halophilic *Haloferax mediterranei* has been largely investigated for its ability to produce the copolymer Poly(3-hydroxybutyrate-co-3hydroxyvalerate) (PHBV) using organic waste as carbon sources and in high-salinity environments without requiring sterilization. Different authors studied genes and pathways responsible for PHBV production, thus allowing the identification and regulation of fermentation parameters for enhancing cell growth and increasing PHBV accumulation.
Different food waste has been tested for PHBV production by *Haloferax mediterranei*. Among them, the whey from cheese production and starch-based byproducts have been widely investigated. The use of whey required a-galactosidase treatment to hydrolyze the lactose and the addition of trace element solution (SL6) to obtain a final concentration of PHBV of 1.18 g/L. The production of wasted bread-derived substrate was moreover optimized including seawater instead of the SL6 to reduce the costs for media supplementation. In this case the production under optimized conditions of fermentation was 1.53 g/L. Both productions were conducted in a 3-liter bioreactor. However, the amount of biopolymer produced is not the only parameter to assess. The characterization of PHBV and the importance of hydroxyvalerate (HV) abundance in the formation of PHBV copolymer is usually evaluated to assess the properties of this biopolymer and the downstream processing options can influence this composition. Nowadays several crucial factors associated with industrial scale-up still need to be evaluated at industrial scale to obtain a sustainable and economic alternative to the other biopolymers using inexpensive food waste and *Haloferax mediterranei*.

Acknowledgements
This study was carried out at the Agritech National Research Center, and it received funding from the European Union Next-GenerationEU (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR)—MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.4—D.D. 1032 17/06/2022, CN00000022).
This manuscript reflects only the authors' views and opinions; neither the European Union nor the European Commission can be considered responsible for them.

Plant growth-promoting bacterial consortia isolated from halophytes to improve crop response to salinisation and climate change

Racioppo, Angela[1]; Guerrieri, Angela[1]; Speranza, Barbara[1]; Botticella, Lucia[1]; Conversa, Lucia[1];Corbo, Maria Rosaria[1]

[1] *Department of the Science of Agriculture, Food, Natural Resources and Engineering, University of Foggia, Foggia, Italy*

Salinisation of soils is one of the main biotic stresses impacting on agricultural lands [1]. The use of beneficial bacteria adapted to high salinity conditions is a viable option for improving crop production in salinity affected areas, given that saline soil reclamation is a complex process. Halotolerant plant growth promoting bacteria (PGPB) are able to enhance plant growthby increasing soil carbon, nitrogen and mineral availability and uptake [2].
Over the years, coastal and saline regions, including the area of Margherita di Savoia saltworks, north-eastern Apulia (Italy), have proven to be a natural source of beneficial microbes adapted tohigh salinity. The area of Margherita di Savoia saltworks is said to have a unique, diverse and richindigenous microbiota that is still largely unexplored.
Therefore, the aim of the present study was to isolate, characterise and select potential PGPB from *Cakile maritima* plants collected in two sites located in Margherita di Savoia. In particular, the microbiological sampling was carried out in triplicate at three phenological stages (seedling, vegetative growth, flowering) of plant life cycle, during the year 2023. A total of 180 (150 rhizobacteria and 30 endophytes) bacteria were isolated and characterised by using morphological, biochemical, and molecular approaches.
The halotolerant isolates which possessed plant growth promoting traits including phosphate, andsilicon solubilization, indole acetic acid, and siderophores production, ammonia generation, drought and salt resistant were selected. Further, the effect of three halotolerant isolates which showed most prominent PGPB activities, and genotypically identified, was evaluated in a growth chamber on
C. maritima plants under high salinity conditions. The halotolerant isolates improved the plant growth especially under moderate saline conditions in comparison to non-inoculated control plants.

References

[1] Mukhtar, S, Malik KA, Mehnaz S. (2019). Microbiome of Halophytes: Diversity and Importance for Plant Health and Productivity. Microbiology and Biotechnology Letters, 47(1), 1–10.
[2] Racioppo A, d'Amelio A, De Santis A, Bevilacqua A, Corbo MR, Sinigaglia M. (2023). Potential Use of Plant Growth-Promoting Bacteria to Enhance Growth and Soil Fertility in Marginal Areas: Focus on the Apulia Region, Italy. Agronomy, 13(12), 2983.

Acknowledgments

This study was carried out within the: Agritech National Research Center and received funding from the 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,CN

Genomic and metabolomic characterization of a new toxic cyanobacteria

Cordeiro, Rita[1]; Luz, Rúben[1]; Fonseca, Amélia[1]; Gonçalves, Vitor[1]

[1] *CIBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, InBIO Laboratório Associado, BIOPOLIS Program in Genomics*

Cyanobacteria genomes and genomic analysis have been increasing in recent years since genomics allows for more complete studies and a deeper understanding of several matters, such as taxonomy and secondary metabolites bioprospection. A previous work on BACA's (Bank of Algae and Cyanobacteria of the Azores) cyanobacteria diversity, based on 16S rRNA phylogenetics, revealedthe first evidence of the high and undescribed diversity of cyanobacteria in the Azores islands. This work reported four toxic strains, two cylindrospermopsin producers (by LC-MS/MS), BACA0025and BACA0031, with 16S rRNA phylogenetics indicating it as a possible new genus. Here, we aimed to produce and explore these strains genomes for a deeper characterization. Genomes were sequenced through Illumina and, assembled and annotated following Luz et al. [1]. Genomic characterization was based on phylogenomics, average nucleotide identity (ANI), average amino acid identity (AAI) and biosynthetic gene clusters (BGCs) identification. Metabolomic analysis was assessed through HRLC-MS. Phylogenomics, ANI and AAI revealed evidence that strengthened the description of a new genus within the Aphanizomenaceae family. Identification of BGCs revealed several secondary metabolites besides the previously identified cylindrospermopsin [2], such as Anabaenopeptins and heterocyst glycolipids. The comparison of BGCs betweenproducing cyanobacteria also allowed a deeper characterization. The metabolomic analysis confirmed metabolites identified by genomics, such as Anabaenopeptins and the Cylindrospermospin analog. This work confirms the rise of a new toxic cyanobacteria genus, isolated from several freshwater lakes in Pico island (Azores. Portugal), producer of secondary metabolites of interest, thus highlighting the uncovered cyanobacteria diversity in the Azores and its biotechnological potential.

References

[1] Cordeiro R., et al. 2021. Cyanotoxin screening in BACA culture collection: identification of new cylindrospermopsin producing cyanobacteria. Toxins 13: 258.

[2] Luz R., et al. 2023. Description of *Pseudocalidococcus azoricus* gen. sp. nov. (Thermosynechococcaceae, Cyanobacteria), a rare but widely distributed coccoid cyanobacteria. Diversity 2023, 15, 1157.

Acknowledgments

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Microorganisms for the environment: Technological robustness of Plant Growth Promoting Bacteria for Mediterranean crops

d'Amelio, Annalisa[1]; De Santis, Michele[1]; Bevilacqua, Antonio[1]; Flagella, Zina[1]; Sinigaglia, Milena[1]; Corbo, Maria Rosaria[1]

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Soil degradation caused by climate change could threaten economically important crops, such as durum wheat, especially in the Mediterranean regions where climatic and meteorological conditions are strong contributors. The aim of modern agriculture is therefore to find sustainable solutions to optimise the biological and economic productivity of the soil resources. Plant Growth PromotingBacteria (PGPB) are the most promising approach to achieve these goals, as they offer a wide range of benefits in agriculture, including increasing crop productivity, improving soil nutrient levels and restoring soil fertility [1]. However, to use them properly, it is necessary to understand their limits, for example, whether they can be applied in different conditions from those in which they were isolated and developed. Thus, this research aims at assessing the technological robustness of some PGPB strains, focusing on the resistance to commercial fungicides, adhesion to seeds to design a protocol for bacteria use in field, growth profiles as a function of pH and temperature, persistence in soil also under extreme conditions.
The strains were generally resistant to fungicides; in addition, the adhesion performance was >80% and for many microorganisms at 95-98%.
Concerning the persistence in soil, they show a prolonged viability, although the increase of temperatures (at 45°C) could be a challenge.
Generally, the results show a high technological robustness of the strains and their potentiality asactive ingredients of bioformulates.

References

[1] Racioppo, A.; d'Amelio, A.; De Santis, A.; Bevilacqua, A.; Corbo, M.R.; Sinigaglia, M. (2023) Potential Use of Plant Growth-Promoting Bacteria to Enhance Growth and Soil Fertility in MarginalAreas: Focus on the Apulia Region, Italy. Agronomy, 13, 2983.

Microbial characterization of endophytic bacteria from halophytic plants

Guerrieri, Angela[1]; Speranza, Barbara[1]; Racioppo, Angela[1]; Giancaspro, Angelica[1]; Lotti, Concetta[1]; Corbo, Maria Rosaria[1]

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Halophytic plants are adapted to harsh environmental conditions and can grow in coastal areas known for their high salinity. The physiology and metabolism of such plants are influenced by their microbiome, which includes a specific class of microorganisms: Plant Growth-Promoting Endophytic bacteria (PGPE), a heterogeneous group of microorganisms that inhabit the interior of plant tissues and are known for their beneficial effects on growth and health of the host. PGPE facilitate plant development by several mechanisms similar to the rhizosphere bacteria, includingfixation of N2, solubilization of phosphate, production of indole acetic acid and siderophores, andgreater availability and uptake of carbon, nitrogen, and minerals from the soil. The identificationof endophytic microorganisms appears to be an important tool for developing sustainable agriculture and improving the growth and stress tolerance of crops in marginal areas.
This study aimed to isolate and characterize endophytic bacteria from two halophytic plants: *Cakile maritima* Scop. *and Salicornia europea*, collected in the Margherita di Savoia saltworks, in thenorth-eastern Apulia (Italy). The isolation of 40 endophytes was carried out according to Christakis et al. [1]. All isolates were characterized by phenotypic (Gram staining, catalase, oxidase, and urease) and technological tests (phosphate solubilization, ammonium production, nitrification, siderophore production, drought and salt resistance, and silicon solubilization). DNA barcoding based on 16S rRNA sequencing was used for the identification of the isolates.
The obtained results suggest that halophytic plants harbour a variety of putative endophytic bacteria which exhibits tolerance to NaCl and displays different plant growth-promoting traits. Our findings suggest the possible implications of these PGPE in improving the quality and productivityof halophytes in saline soil and in protecting other important plants against salt stress.

References

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Microbial collection from vegetable compost tea to developbacterial consortia suitable for plant disease control

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Compost teas (CTs) are fermented aqueous extracts obtained from vegetable compost by extraction and/or fermentation processes in liquid phase. They are rich in organic and inorganic molecules active as plant biostimulants, and in microorganisms capable to counteract phytopathogenic fungi and bacteria. About 5% of the total CT microflora is cultivable in vitro: the most represented genera are *Bacillus*, *Pseudomonas*, *Serratia*, *Stenotrophomonas*, *Aspergillus*, *Flavobacterium*, *Fusarium*, *Gliocladium*, *Penicillium* and *Trichoderma*. The PLANTìA project, funded by AGER Agroalimentare e Ricerca (Ager Project – Third Edition), aims to select beneficial microorganisms fromtwo vegetable CTs. One hundred isolates extracted from these CTs are characterized for their biocontrol activity against four phytopathogenic fungi of leguminous and grapevine crops: *Rhizoctonia solani* (RS) CREA OF 1333.1, *Macrophomina phaseolina* (MP) CREA OF 373.2, *Fusarium solani* (FS) 897.1 and *Botrytis cinerea* (BC) CREA_OF 1531. Preliminary results showed thatthe growth of RS appeared inhibited by 4 isolates, FS by 13 and BC by 6. MP growth was slightly affected by 10 isolates. The effective antagonistic bacteria were identified as Pseudomonas. Theywill be further characterized for species identity, motility, production of biofilm, indole acetic acid,protease, siderophores and volatile compounds, ability to fix nitrogen and to solubilize phosphate. Moreover, tolerance to extreme pH, salinity and temperature will be determined to select bacteria able to protect plants under unfavourable environmental conditions. The most promising accessions will be used for in vivo biocontrol assays. This work will allow to implement microorganism collections, to preserve microbial biodiversity and to detect microbial features related to the effectiveness of a microorganism in protecting the plants from biotic and abiotic stress factors. The design of new effective microbial consortia is the final objective of the research.

Dietary microbes and Mediterranean diet

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The Mediterranean diet, characterised by high consumption of fruits, vegetables, whole grains, legumes, and olive oil, alongside a moderate intake of fish and poultry, has long been associated with numerous health benefits, including reduced risks of cardiovascular diseases, cancer, and neurodegenerative disorders. Emerging trends in scientific research highlight a contribution of dietary microbes in human health and nutrition. Our research synthesises current evidence on the microbial content of Mediterranean diet components, such as fermented dairy products, olives, vegetables and fruits, in order to define appropriate microbe-depleted and microbe-rich diets for future experimental trials. The research explores the microbiological classification of food items within the Mediterranean diet and examines 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. The different food items were categorised according to microbial content: low (Lo), 10 CFU/g. This classification revealed significant variations in terms of microbial abundance, providing the basis for a critical evaluation of this aspect. Subsequently, we established two isocaloric daily diets, both compliantwith the guidelines of the Mediterranean diet: one microbe-depleted and one microbe-rich diet. By starting with similar products, we highlighted how food processing and ingredient choices can modify dietary microbe load, contributing to design diets for specific experimental proposes. Our findings emphasize the need for further research to enhance health benefits from microbial intake associated with the Mediterranean diet, offering promising directions for future studies in food science and nutrition.

Acknowledgments
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 within 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.

Fungi as bioresources for remediation of HCH-contaminated soils: from microbial community-level physiological profile to selective isolation in enrichment

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The interaction between human activities and global change (including persistent chemicals pollution) poses severe threats for the soil microbiota thus reducing the provision of ecosystem services1. In this context -, -, and -hexachlorocyclohexane (HCH) are highly persistent organic pollutants ofglobal concern, and a severe risk for human health and ecosystem functioning. Soil fungi, thanksto the ability to tolerate, bioaccumulate and biodegrade HCH, are important bioresources as biobased solutions for HCH-contaminated soil remediation. The study area was selected within the National Priority Site “Bacino del Fiume Sacco” in the Metropolitan City of Rome (Italy). Soil cores, up to 1 m of depth, were collected from 2 plots and later divided in topsoil (TS: 0-10 cm) and subsoil (SS: 10-100 cm) samples. The first goal was to characterize the microbial community level physiological profile, so the soil samples were analysed by the Biolog EcoPlateTM Technique2 to compare metabolic activities of the communities at different depths (TS and SS). Moving on, the project focused on the fungal fraction of the microbial community, evaluating the fungal loaddifferences between TS and SS, through the count of the colony forming units (CFUs/dry soil weight). The CFUs results show a higher fungal load in topsoil than that in subsoil by one order of magnitude. To isolate fungal bioresources suitable for HCH degradation, a selective enrichment procedure with a high concentration HCH mixture as the only carbon source, was carried out. At the end of the procedure several species, mainly belonging to Fusarium and Alternaria genera,were isolated and are currently preserved in the Culture Collection of the Fungal Biodiversity Laboratory (FBL) of the Department of Environmental Biology of Sapienza University of Rome. The isolated fungi represent useful bioresources for further studies aimed at the development of mycoremediation application for HCH contaminated soil remediation.

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From culture collections to biorecovery of strategic elements: exploring fungal interactions with Germanium and Indium

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Fungi in nature play crucial roles in geology and ecology, and represent valuable resources for sustainable recovery processes1. Fungi can tolerate, bioaccumulate, and transform toxic compounds and several strategic elements, e.g. germanium (Ge) and - indium (In). These are elements of fundamental interest in high-tech products, and their extraction often heavily impacts ecosystems.Their fungal-mediated recovery from electronic wastes offers an eco-friendly solution to mitigate environmental impacts and support sustainable resource management2.
In this study 24 fungal isolates were selected based on available literature on fungi-element interactions, known metal-fungi mechanisms, and biological and ecological features. These fungi were chosen among those preserved in the culture collections of the Fungal Biodiversity Laboratory at Sapienza University of Rome and CNR mycology laboratories. Since siderophores are crucial in metal-fungi interactions, the selected fungi were screened for their production through the ChromeAzurol-S (CAS) assay3. Moreover, considering the importance of laccase in bioleaching processesand the fact that these elements share chemical features with those playing primary roles in fungal biology, a screening to evaluate the production and release of laccase was carried out through theGuaiacol assay 4. Finally, fungal tolerance to Ge and In was evaluated using exposure to 5 mM Ge as GeO_2 or 2.4 mM In as In_2O_3. The screenings were carried out on solid Raper Hoffman medium for 14 days at 25°C. The Rt:Rc (%) index, based on colony areas, and the T.I. (%) index, based on dry weights5, were calculated to evaluate fungal tolerance. The variation in medium pHwas also assessed.
Most of the strains were able to produce siderophores, with 7 showing high response, whereas laccase release was only detected in 8, all basidiomycetes. Finally, all fungi were able to grow in all tested conditions, even though several evident Ge/In effects were observed.

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Microbial consortia the great bio-based solution for the bioremediation of co-contaminated soils: study of microbial resources isolated from a decommissioned military site.

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It has been estimated that around 33% of global soil is degraded1. Considering estimations of soilloss and of contamination effects exacerbation due to future climate change scenarios, the need for sustainable remediation of contaminated soils is crucial for maintaining the ecosystem services.
This study investigated fungal and bacterial strains, isolated from soils and a *Plantago lanceolata*'s rhizosphere sampled in a decommissioned military site, for their potential in detoxificationof co-contaminated soil. Globally 101 fungal taxa and 185 bacterial strains were isolated from the samples and are currently preserved in the Fungal Biodiversity Lab. culture collection. To select the most suitable bioresources, all these isolates were screened in a microwell plate enrichment experiment2 for their ability to utilize PAHs as their sole C source also in co-contaminationwith Pb and/or Zn. 13 fungal species and 15 bacterial strains were able to grow using PAHs asC source also in co-contamination conditions. So, as microbial consortia are reported for their higher bioremediation efficacy3, in-vitro compatibility tests4 were performed among the selected strains isolated from the same sampling plot to define the consortia formulations. The 9 resultingconsortia and an additional one formulated with 5 Trichoderma strains, were tested in microcosm conditions to assess their effectiveness in detoxifying soil polluted with PAHs, PAHs+Pb, PAHs+Zn, PAHs+Pb+Zn. The detoxification efficacy of the biological treatments was evaluated by an array of ecotoxicological assays, whose results revealed that, despite several consortia successfully reduced the toxicity in at least few contamination conditions, consortium D was the mosteffective in reducing toxicity in the microcosm experiment. Consequently, the consortia D and A are currently being studied in a pot experiment, including also a microbially assisted phytoremediation condition with *P. lanceolata*, to confirm their efficacy.

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The CABI Biological Resource Collections: evolving to meet the changing needs of users.

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Established in 1947, the CABI Genetic Resource Collection (GRC) is one of the most significant global collections of microorganisms, containing >28,000 fungi and approximately 2,000 bacteria. The collection is primarily comprised of plant-associated and environmental microorganisms. It contains several smaller collections, including the UK's National Filamentous Fungus Collection, National Collection of Wood-rotting Fungi; the UK Aquatic Phycomycetes Collection, the BritishAntarctic Survey Collection and others. One benefit of the broad geographic coverage of the collection and the lengthy timeframe over which strains have been deposited, is that strains in the collection may be used to study the evolution of key fungal groups, as well as enabling reappraisals of legacy strains, with new molecular tools, which may not only shed light on 'new' cryptic species.But also allow us to understand the relationships between organism groups. Recent studies have included contributing to the major reappraisal of the genus *Fusarium* (being led currently by theWesterdijk Institute) and a study of genome evolution in the coffee wilt fungus, *Fusarium xylarioides* with Imperial College, London.

Recently, the UKRI-funded UK Crop Microbiome CryoBank has established a collection of 36,000 bacteria and 4,800 environmental soil rhizosphere samples, held within the CABI CryoFacility, this extends the collection's holdings to include environmental and pure cultures from several key crops. This builds on CABI's expertise in cryopreservation science and provides links with EU infrastructure projects such as the EU Microbiome (RI) Enabler and EU Microbes4Climate and together enables both a better understanding of the rapidly developing 'microbiome' research area, but crucially supports the broader research community. Such developments are clearly demonstrating the continued relevance of the CABI Collections to cutting-edge science.

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Exploring methodological aspects of microbiota transplantation in black elder (*Sambucus nigra*)

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Plant microbiome studies have previously revealed an impact of endophytic microbial communities on complex plant traits such as disease resistance, phytochemical content, or yield. However, targeted manipulation of plant microbiota to improve plant properties is yet to be achieved. Here weexplore the idea of microbiota transplantation in wild black elder (*Sambucus nigra*) populations in Latvia. We attempt to develop transplantation methods by identifying methodological aspects significantly affecting the preparation and storage of microbiota inoculums. For this research, we explored three methodological aspects – we analysed the effect of fractionation of plant slurry used for microbiota transplantation on the composition of the microbial community, we conducted a long-term storage experiment to see if and how the microbiome inocula changed after storing in the freezer for longer periods, and lastly, we tested two different microbiota application techniques
– injecting with a syringe and spraying.
Wild samples of unripe black elderberries were collected and surface-sterilized in the lab. Samples were disintegrated, divided into sub-fractions based on particle size, and stored at -80°C. For further analysis, total DNA was extracted, and microbial communities were characterised using target loci amplicon next-gen sequenced. We used the sequence data of ITS and 16S (v1 – v6 regions) ribosomal RNA gene sequences to characterize the endophytic microbiome.
The preliminary results show a slightly increased bacterial diversity in plant slurry samples composed of particles ranging in size from 57 to 165 m. The proportions of the main bacterial generafound in filtered samples also differ between larger-sized particles (57-165 m) and smaller-sized particles (0-57 m). Our research prospects are to optimize and implement in practice plant microbiota transplantation methods, which could potentially be applied in other economically important plant species in the future.

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Plant growth promotion endophytes from sicilian native grapevines as plant biostimulants for climate-ready crops

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Mediterranean crops are undermining by increasingly extreme weather events in particular heat waves, drought and intense rainfall. Water scarcity has become the most limiting factor of agriculture in the Mediterranean regions and the expansion of arid areas is threatening traditional productions, such as viticulture. To improve the resilience and sustainability of vineyards, the endophytic microbiota of the Phyllosphere was studied in native grapevine cultivars adapted to arid conditions, and in wild grapevines from Sicily.
Endophytic communities were isolated and identified by sequencing of 16S rDNA region. The most representative isolates, belonging to species already known for their Plant Growth Promoting (PGP) properties were characterized by in vitro assays. The tested PGP properties included indole-3-acetic acid and siderophore production, phosphate solubilization, nitrogen fixation, biofilm formation, the presence of 1-aminocyclopropane-1-carboxylic acid deaminase gene and of enzymatic activities. Among the 13 PGP activities tested, more than 70% of the strains exhibited five to sevenPGP traits concurrently. In vitro tests showed that most of the isolates were able to stimulate plant nutrition and growth, through biofilm formation, amylase and lipase activity, siderophore production and nitrogen fixation. Two bacterial consortia combining three distinct strains were selected to cover the greatest number of PGP properties. The potential role of each consortium inpromoting plant growth and drought tolerance was tested on an indicator plant (*Medicago sativa*)both in vitro using drought resistance tests and in pots under controlled greenhouse conditions. Plant physiological status was determined by measuring plant growth and photosynthetic parameters. The use of native microbial consortia is becoming a promising technique to reduce the harmful impacts of chemical fertilizers and contribute to a more sustainable agricultural system,to cope with climate change.

Exploring sicilian olive tree eco-friendly endophytes for sustainable agriculture

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Olea europaea L. is one of the most important crops that characterize the Mediterranean agriculture. Olive trees can withstand various environmental stresses such as heat, drought, salinity, andhigh levels of UVB, by implementing a variety of adaptations strategies including the morphological changes and the activation of physiological and biochemical mechanisms that make it a resilient woody crop. Olive tree microbiota comprises endophytes, microorganisms that promote plant growth and stress tolerance. To develop a microbe-assisted improvement of production, and agroecosystems resilience, the Phyllosphere bacterial communities in three olive cultivars and wild olive trees from Sicily were investigated over four phenological stages. The isolated and molecular identified microorganisms were characterized to assess the presence of plant growth promoting (PGP)traits. The tested PGP properties included indole-3-acetic acid and siderophore production, phosphate solubilization, nitrogen fixation, biofilm formation, the presence of 1-aminocyclopropane1-carboxylic acid deaminase gene and of enzymatic activities (DNase, amylase, cellulase, lipase, chitinase, protease and pectinase). All isolates showed at least one of the screened PGP propertiesand more than 60 % of them exhibited from five to ten PGP traits concurrently. The most common PGP properties were siderophore production, biofilm formation and nitrogen fixation. Based on PGP activities, two different endophytic consortia were formed and tested for in vitro compatibility and drought assay. The potential role of bacterial consortia to promote the growth and the tolerance to drought of an indicator plant (*Medicago sativa*) was assessed in pots in controlled greenhouse conditions. Plant growth and photosynthetic parameters were measured to determine the plant physiology status. Applications of native microbial consortia could be thought for a sustainable agriculture capable of facing the new climate scenarios.

Fungal diversity in the precious Mediterranean red coral *Corallium rubrum*

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Corals maintain a symbiotic relationship with a variety of microorganisms, including bacteria and microeukaryotes, which inhabit their tissues and provide important nutrients. These microeukaryotes include various groups of protozoa, microalgae, and fungi. However, fungi, an often overlooked but integral component of these symbiotic communities, may play a critical role in coral health and resilience to stress. In this work, we have characterized the diversity of cultivable and non-cultivable fungi associated with the precious red coral *Corallium rubrum* that together with other gorgonians,is part of the Marine Animal Forests (MAFs) harbouring an incredible biodiversity in the Mediterranean Sea. For this purpose, we collected 6 coral nubbins at 40m depth in Villefranche-sur-mer,(Ligurian Sea). By coupling culture isolation and metabarcoding techniques, and separating the host tissue from the skeleton, we observed a large fungal biodiversity This comparative approach showed that the combination of dependentand independent-culture methods is essential to reduce bias and obtain a more accurate representation of the fungal community. Our findings underscorethe importance of understanding the diversity and ecological roles of microeukaryotes. This is critical for coral conservation and management, especially in the face of anthropogenic threats and climate change.

A network-based approach to elucidate the dynamics of the native microbiota's response to a multi-kingdom microbial inoculant in raw brewer's spent grain

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Identifying the key factors that influence microbiota stability and resilience is of uttermost importance for effectively managing microbial resources in various environmental applications [1]. Indeed, microbial inoculants, typically chosen under controlled conditions, often struggle to survive in the field due to factors like soil type, nutrient levels, environmental stress, and competition with native microbiota [2,3]. Recently, the interactions between a multi-kingdom microbial inoculant and the native microbial population of untreated brewers' spent grains (BSG) were assessed [4,5]. Specifically, inoculated and spontaneous solid-state fermentation (SSF) processes were monitored for 90 days by analyzing BSG chemical and biochemical properties as well as the evolution of the microbiota through high-throughput sequencing of 16S and ITS rRNA. Starting on day 14, the bacterial and fungal genera present in the bioinoculant were no longer able to survive in the BSG andwere outcompeted by the native microbiota. Nonetheless, the bioinoculant had a significant impact on the chemical, biochemical and fertilizing properties of BSG. Cross-kingdom co-occurrence network analysis suggested that this impact was associated with the reshaping of the native microbial population in the inoculated samples. Indeed, the network from the inoculated samples was more connected and stable due to stronger and more significant correlations. Interestingly, most of these interactions were positive, suggesting mutualistic relationships between microbial taxa. These findings suggest that comprehending the interplay between native and external microbial communities in complex ecosystems, and employing microbial inoculants tailored to specific environments or conditions, facilitates the creation of effective bio-based applications. Consequently, the microbial strains with agronomic significance, derived from the native microbiota of BSG, have been archived in the MBDS-UNISSCC culture collection.

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Exploring the oenological characteristics of different non-*Saccharomyces* species in grape must fermentation.

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Complex biochemical processes occur in wine fermentations, where yeasts, specifically Saccharomyces cerevisiae, convert sugars to ethanol, carbon dioxide, and other volatile compounds. Nowadays, the role of non-*Saccharomyces* yeasts in winemaking is gaining significant interest due to their ability to enhance the aromatic complexity and sensory profile of wines.
In this work, thirty-five yeast species from the CREA-Collection of Microorganisms of the Viticultural and oenological Environment (CMVE), conserved at CREA-VE in Asti, were tested. Yeasts were inoculated in wine must, and fermentations were monitored by weight loss. At the end of alcoholic fermentation, the following parameters were analysed: alcoholic degree, residual sugars, malic acid, lactic acid, volatile acidity, and glycerol.
The results showed that only three species completed alcoholic fermentation, with residual sugars less than 1 g/L: *Saccharomycodes ludwigii*, *Schizosaccharomyces pombe*, and *Zygosaccharomyces bisporus*. Regarding the volatile acidity, most of the tested species produced below 1 g/l of acetic acid. Some yeasts, in particular *Hanseniaspora osmophila*, *Hanseniaspora valbyensis*, *Hanseniaspora uvarum*, *Zygosaccharomyces rouxii*, *Zygosaccharomyces bailii*, *Pichia terricola*, and the already-known *Schizosaccharomyces pombe*, could reduce malic acid.
Interesting data were found for *Kazachstania exigua*, which exhibited high glycerol production and high consumption of the sugars present in the must.
This study underlines the importance of discovering the distinctive characteristics of the yeast species. This could facilitate to find interesting yeasts that are currently not employed in wine fermentation. Non-*Saccharomyces* species as starter cultures are becoming an increasingly important approach in modern winemaking; they can contribute to producing specific aromas or be used tomake wines with reduced ethanol concentrations.

Harnessing Microbiomes for Drought-Resilient Agriculture: Insights from the BIOMEnext Project

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The global increase in water scarcity presents a major challenge to sustaining crop productivity. Lack of adequate water results in the degradation of the photosynthetic apparatus, interruptionsin essential metabolic processes, increased production of free radicals, and compromised plant root structures. Drought is a key stress factor that directly affects the osmotic balance of plant cells. Within the BIOMEnext Project, innovative, composite, and eco-friendly farming systems have been evaluated to improve the resilience of Mediterranean fruit farming to climate change. Specifically,metagenomic analyses were conducted, characterizing the core rhizosphere and endophyte microbiomes and their predicted functions in four olive varieties (Arbequina, Chemlal, Koroneiki, and Shengeh) under wet and dry conditions during a one-year field experiment. DNA from both rootendophytes and soil rhizosphere was extracted and sequenced using Oxford Nanopore technology, producing long reads of the 16S rRNA gene. These long reads enabled species-level identificationof the microbial composition using the Emu tool. Additionally, a novel tool was developed for functional annotation using PICRUSt2 with long reads. A focus was placed on functions related to cold-heat stress, general stress and heat-shock, which are all functions often associated with drought resistance. Finally, PGP functions were also included in the research. This research allowed the identification of genera associated with these functions which, although not differing much at the functional level between the rhizosphere and roots, are completely different at the genus level. In fact, two core microbiomes has been defined, one for rhizospheric soils and one for roots, which are potentially involved in drought resistance.

Acknowledgements
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Harnessing Fungi for Eco-Friendly Biosurfactant Production: joint efforts from CECT and MUT collections to unveil this potential among the preserved strains

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Biosurfactants are gaining significant attention in the global market as natural additives for commercial products and environmental applications due to their ability to reduce surface and interfacial tension between fluid phases. Compared to chemical surfactants, biosurfactants offer enhanced stability across a range of physico-chemical conditions and are environmentally friendly. The biotechnological production of biosurfactants is rapidly evolving, with fungi emerging as highly promising producers of these valuable biomolecules.

In this context, the aim of this work is to explore the potential of fungal biosurfactant productionthrough various screening tests on 20 yeast strains. These strains were selected from the most representative genera deposited in the Spanish Type Culture Collection (CECT) and Mycotheca Universitatis Taurinensis (MUT) mainly isolated from wastewater sediments of olive oil mills.

The screening involved: the oil displacement test and the drop collapsing assay. The fungi were pre-grown in 100 mL flasks containing 40 mL of modified mineral salt medium (MSM) with oliveoil (50 mL/L) as the sole carbon source to stimulate surfactant production. The flasks were incubated at 24 °C with agitation at 120 rpm for 21 days. The capability to produce biosurfactants was tested at four time points (T3, T7, T14, and T21).

The results indicate that four strains belonging to the species *Wickerhamomyces anomalus* were the best performers in both screening tests revealing their capability to produce strong biosurfactantsand powerful emulsification activities. Ongoing analyses are trying to elucidate the metabolites and the metabolic pathways involved. However, preliminary results based on Phenotype Microarray (PM, Omnilog) highlight the activation of similar metabolic pathways between the four strains of *W. anomalus*, which are distinct from those of the other strains tested.

Biotechnological production of biosurfactants from fungi represents a promising, simple, and cost-effective method for large-scale production, potentially replacing synthetic surfactants in various industries. Further works are necessary to identify the secretes and to scale-up their production.

Acknowledgements
Part of this work was granted by the European Commission—NextGenerationEU, Project "Strengthening the MIRRI Italian Research Infrastructure for Sustainable Bioscience and Bioeconomy", code n. IR0000005.
Marta Gumiel is recipient of INVESTIGO and ERASMUS+ grants.

Changes in bacterial, archaeal, and fungal communities from saline soils cultivated with tomatoes in Italy

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Soil salinization is a major concern in modern agriculture. In southern Italy, the climate change scenario is challenging tomato crops, especially because of increased average temperature, irregular precipitation patterns, and scarcity of irrigation fresh water. Apulia ranks among the top Italian regions producing processing tomatoes, but certain areas are being subjected to salinization, especially near the coast. A metagenomic study was conducted to compare the microbiome from saline and non-saline soils in the Apulia region. Three areas, each including four fields with threereplicated samples, were sampled at three time points during the tomato cropping cycle, viz. post transplanting, mid-season, and near-harvest time, for a total of 108 soil samples. Physical-chemical analysis was carried out to characterize the soils, and DNA was extracted for Illumina sequencingof amplicons specific to bacteria, Archaea, and fungi.
Saline and non-saline soils differed for exchangeable sodium, limestone, cation exchange capacity, and some macro and micro-nutrients, whereas no significant difference occurred in the texture. Electrical conductivity increased during the cropping cycle in fields irrigated with saline water, suggesting that soil salinization depended on irrigation water quality rather than pedoclimatic conditions.
The amount of DNA extracted from soil samples, as well as the number of sequencing reads, wereunexpectedly low at post-transplanting compared to the other two time points, indicating that the establishment of microbiomes in the rhizosphere took place as the crop had developed. In thethree considered kingdoms, i.e., Bacteria, Archaea, and Fungi, differential operational taxonomicunits (OTUs) were identified in saline and non-saline soils, thus taxa with a better fitness under high salinity were identified. This paves the way for searching for new plant-beneficial microbes sustaining tomato crops cultivated in salinized soils.

Acknowledgements
This work was funded by the research project “Deciphering microbiomes from extreme environments for the improvement of crop resilience to climate change, food security and safety (MiCroResi)” (framework “4th EU-LAC Multi-thematic Joint Call for collaborative projects from Europe, Latin-America and the Caribbean Countries”) and by “Agritech National Research Center and received funding from the European Union Next-GenerationEU” (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR), MISSIONE 4 COMPONENTE 2 INVESTIMENTO 1.4, D.D. 1032 17/6/2022,CN00000022). This manuscript reflects only the authors’ views and opinions,neither the EU nor the European Commission can be considered responsible for them.

A polycyclic aromatic hydrocarbons (PAHs) contaminatedsoil as source of microorganisms for bioremediation purposes

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The soil ecosystem is variable and complex and hosts a huge variety of microorganisms, includingfungi that provide several ecosystem services.
Due to the increase of industrial activities and to the combustion of fossil fuels and organic matter,the release of PAHs threatens the health of natural ecosystems. Soil contamination by PAHs is an ever more severe issue that requires a sustainable solution. A wide range of fungi is capable ofdegrading these recalcitrant compounds through their enzymatic arsenal. Thus, investigating andidentifying the cultivable mycobiota inhabiting PAH-contaminated soils is crucial for designing appropriate remediation approaches.
In this work, we investigated the fungal diversity of a formerly cultivated urban garden in Torino(Italy), mainly contaminated by benzo(a)pyrene, benzo(g,h,i)perylene, fluoranthene, and phenanthrene.
Overall, 181 fungal isolates, mostly belonging to Ascomycota were retrieved and were affiliated to 38 genera and 66 species, including putative novel species affiliated to genus Coniochaeta. In parallel, a culture independent approach based on the sequencing of ITS2 region, applied to investigate the fungal diversity, confirmed the dominance of Ascomycota (99%), while Basidiomycota and Mortierellomycota accounted for less than 1%.
Following, to select those strains capable of degrading PAHs, enrichment assays were conducted with the target pollutants as sole carbon source. More than 99% of the 104 isolates retrieved belonged to Ascomycota, while only one Basidiomycota was isolated in axenic culture. The selectionapplied allowed the development of a number of species (61%) that were not detected in the bulksoil.
The fungi derived from the enriched cultures are currently objects of deep investigation whose outcome will serve to evaluate their degradative potential and to develop remediation strategies for in situ applications.

Acknowledgments
Bando BRiC – edizione 2022 – ID:65 Tecniche avanzate di micorimedio: una soluzione sostenibile per la bonifica di suoli contaminati e per la sicurezza per gli operatori CUP: D13C22003330005

Impact of newly developed biofertilizer “polyazorich” on soil agrochemical properties during crop cultivation

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The development of methods for restoring the natural balance of soil microflora, the use of different preparative complexes separately and in various compositions are of great importance foragricultural farming in order to revive degraded soils. The use of preparations on the basis of microorganisms is the most effective way to increase the productivity of plants, which makes it possible to preserve the soil fertility and the ecological balance of soils.
The new biofertilizer contains association of most promising domestic free-living (*Azotobacter chroococcum*), symbiotic nitrogen-fixing (*Rhizobium* spp., *Mesorhizobium ciceri*, *Bradyrhizobium japonicum*, *B. arachidis*) and phosphate-solubilizing (*Paenibacillus polymyxa*) bacteria, which areable to convert soil nutrients into a soluble form available for absorption by plants, increase yields,have fungicidal properties.
Agricultural crops were grown in light loamy soil of brown semi-desert type, characterized bya relatively low humus content (2.73%), salinity (0.092%), concentration of carbonates (0.67 mgEq/100g) and sulfates (0.07 mg-Eq/100g), indicators of basic soil nutrients: mobile form of nitrogen (NO_3^-) 30.2, immobile and mobile forms of phosphorus (P_2O_5, PO_4^{3-}) – 10.4, 14.0, respectively and immobile and mobile forms of potassium (K^+) 14.7 mg-Eq/100g.
After biopreparation impact, the organic nutrients content in the soil reached 5.79% within 60 days vegetation. At the same time, there is an increase in NO_3^- to 79.1 mg-eq/100g, as well as saturation of the soil with N_2 up to 16.2 mg/100g. Meanwhile, a decrease is observed in the content of phosphorus salts P_2O_5, PO_4^{3-} and K^+ to 2.86, 3.82 and 5.86 mg-Eq/100g, respectively.

Acknowledgements
This work was supported by the Higher Education and Science Committeeof RA (Research project № 23EDP-4D003).

Session 2 – Microbes from farm to fork

Wednesday 18th September, 5:00pm

Chairs: De Vero Luciana (Italy), Oivanen Pekka (Finland).

Using microbial diversity or complex communities for valorization of side-streams for food applications

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The global food system significantly impacts the biodiversity and climate, with bovine dairy and meat production being the primary contributors. Today, many European consumers, especially the younger generation, are keen to reduce greenhouse gas emissions by adopting healthier and more sustainable dietary choices, including reduced meat consumption. Reflecting this global trend towards lower animal protein intake, a majority of consumers, now identifying themselves as flexitarians, are open to decreasing their consumption of animal-based products. Currently, market-available starter cultures are optimized for bovine dairy, highlighting a pressing need forimproved starter cultures that can enhance the nutritional value of plant-based dairy alternatives.In this context, we present a novel droplet microfluidics community-level high-throughput screening method to isolate lactic acid bacteria (LAB) strains capable of producing riboflavin in soy-based substrates. This technique has the potential to fortify fermented dairy alternatives with riboflavin.Additionally, recent studies have shown the effects of co-culturing LAB with edible basidiomycetes. This investigation explores the interactions between two LAB strains and their influence on the mycelium of two edible fungal species, *Wolfiporia cocos* and *Laetiporus sulphureus*. Notably, changes in mycelial biomass, color, and morphology were observed, suggesting that co-culturing can functionally modify mycelium for potential food applications. These findings underscore the potential impact of novel microbial diversity and co-culturing on various plant-based food industry applications.

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Acknowledgements

This work was funded by Innomission 3 partnership AgriFoodTure (grant No: 1152-00001B, Innovation Fund Denmark) and by the GUDP grant mycoPROTEIN. Additional funding for Hang Xiao was received from Novonesis. The biodiversity included in the abstract is from DTU National Food Institute Culture Collection (NFICC) (dtu.dk).

Are isolation, identification and preservation of microbial strains still useful for food applications?

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In the last 20 years, knowledge and understanding of complex microbial populations have boostedup thank culture-independent high-throughput sequencing (HTS) techniques. The microbiota of a huge number of foods, related environments and processes have been analyzed improving the definition of microbial complexity of samples that is not possible to achieve with traditional microbiological investigation approaches.
For example, in milk and cheeses, several studies confirm that their microbiomes are usually dominated by lactic acid bacteria. However, supplementary information and data report the presence of unexpected, or new, microbial species.
Thus, the question in the title is the daily challenge for food microbiologists.
In the last three years, over than 140 isolates, mainly belonging to the species *Streptococcus thermophilus* and *Limosilactobacillus fermentum*, have been collected from raw milk, natural whey cultures, fresh curd, brined cheese and different cheeses during ripening or cold storage.
In particular, 8 potentially probiotic *Lactococcus lactis* strains and 10 *Lacticaseibacillus paracasei* strains, were isolated by specific protocols from raw milk and Caciocavallo cheese samples, respectively.
In addition to lactic acid bacteria, responsible for milk fermentation and cheese maturation, many other non-dairy species, such as *Klebsiella oxytoca* or *Raoultella terrigena*, were identified.
Thanks to these efforts, 98 bacterial strains were isolated, identified and characterized for their technological properties allowing us to define 4 new dairy processing.
Since these cheeses were realized at dairy plant, at pilot scale level, and will be on the market soon,we could conclude that the improvement of fermented foods can't help but avoid to carry out isolation and characterization of microorganisms from foods and food environments. Overturning the question, are HTS studies really useful for food applications?

Acknowledgements
This study was supported by Italian Ministry of Economic Development within the project "L'evoluzione delle produzioni lattiero-casearie: le biotecnologie valorizzano la tradizione" – ELEVATO n. F/200112/03/X45.
As Scientific Responsible of the project, the Author thanks the efforts made by all CNR-ISPA colleagues allowing to achieve project results here briefly reported.

Soil Health and Agri-Food System Sustainability from a microbiology perspective: A Data-Driven Approach for Agricultural Policy and Practices

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To guarantee the production of healthy and high-quality food, it is crucial to maintain healthy soil, which serves as the foundational bedrock for robust and sustainable agricultural and food production systems. This research explores the vital role of microorganisms in supporting soil ecosystem functions and the broader agri-food chain. Utilizing advanced bibliometric analysis, the study investigates how microbial biomass, enzymatic activity, and community diversity contribute to agricultural sustainability. It emphasizes the importance of microbiological indicators in managing soil health, that is crucial for enhancing the quality and safety of food production from farm to fork. The analysis aims to bridge scientific knowledge on soil microorganisms with practical agricultural applications, assessing how microbial data can inform and improve agricultural policies and practices to ensure sustainable farming and food safety. The study identifies key research trends, gaps, and emerging themes within the scientific community, providing insights into the integration of microbial research into agricultural policy development. Furthermore, it discusses the connections between scientific findings and their practical applications in policy and practice. In conclusion, this study underscores the strategic importance of scientifically validated microbiological indicators in soil management and their key role in the entire food chain, supporting a solid and sustainable 'farm to fork' framework.

Acknowledgements
This research was supported by ECO-READY (GA N◦101084201 https://www.eco-ready.eu) and DELISOIL (GA N◦ 101112855 https://delisoil.eu) projects, funded by the European Union under the Horizon Europe Program. The authors gratefully acknowledge funding from the Italian project Creazione di un HUB italiano a supporto della partecipazione dell'Italia alla Global Soil Partnership ed alla rete di eccellenza europea sulla ricerca sul suolo–SOIL-HUB, granted by the Italian Ministry of Agricultural, Food and Forestry Policies MIPAAF (DM 37072 28/12/2018) CUP C52F18000200006, and the European Union's Horizon 2020 research and innovation programme under GA N◦. 652615 (European Joint Programme SOIL).

Be a QPS or not to be, that is the Quirky Paradox of Safety

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Currently, only microorganisms' species included in the Qualified Presumption of Safety (QPS) list, issued by the European Food Safety Authority (EFSA), based on a generic pre-assessment of security on the available literature and updated every six months, can be intentionally added to food or feed. For lactic acid bacteria (LAB) species not recommended for QPS status due to safety concerns, tests ensuring the absence of antibiotic resistance genes (ARGs) and virulence factors are required before their use in food production (e.g. *Enterococcus faecium*), while for other LAB no specific guidelines are available (e.g. *Streptococcus oralis* and *Streptococcus gallolyticus*). Due to the biodiversity loss in raw matrices and production environments, caused by increasingly stringent hygiene practices applied in manufacturing, starters, mainly LAB, are added to the raw material to be processed to easily and safely carry out the fermentation. Especially natural starters, complex microbial communities having a strain composition mostly undefined and obtained from raw matrices without any microbial selection, are used in artisanal and PDO production of the most typical and high-quality agri-food products, also preserving the microbialdiversity of the production environment. In a recent study, a natural starter obtained from raw ewe milk, without heat treatment application or pro-technological microorganisms selection, included some strains belonging to non-QPS species. Following the current EFSA guidelines, though the strains resulted free from ARGs and virulence determinants, the culture should not be used for food production. Similarly, artisanal manufacturers may not be allowed to produce/use their own natural starter cultures, likely including non-QPS species. An answer to the paradox of non-QPS microbial consortia that are inherently present in raw matrices and natural starter cultures, but do not comply with the EFSA's rules, is needed to use them for food production.

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Investigation on the microbial evolution of cow milk in the passage from stable to mountain pasture, and evaluation of Bitto cheese microbial community

Zago, Miriam[1]; Bonvini, Barbara[1]; Rossetti, Lia[1]; Povolo, Milena[1]; Cabassi, Giovanni[1]

[1] *CREA-Research Centre for Animal Production and Aquaculture, Italy*

Summer transhumance of dairy cows is a seasonal pastoral system practiced in many European countries from ancient times. The microbial composition of milk from three groups of cows, moving from stable in plain farms (PF) to alpine pasture in upper Valchiavenna region (Italy) duringthe summer period (AF) was studied. The AF milk is used to obtain Bitto, a PDO raw milk cheese produced only in summer, when the cows graze on high alpine meadows. Microbiological and metataxonomic analyses were performed to investigate the microbial evolution of 23 milk samples to determine whether the alpine pasture could influence the milk microbiota. Moreover,the microbiota of six Bitto PDO cheese was also studied. The enumeration of lactic acid bacteria (LAB) showed an overall prevalence of mesophilic cocci and of thermophilic cocci and lactobacilli.Within these groups, *Lactococcus lactis* and *Streptococcus thermophilus* were the most abundant,as shown by metataxonomic analysis. A higher total bacterial count in milk from cows in PF compared to those in AF was also detected. Besides, a higher richness of species in AF milk samples compared to PF was observed, showing differences between the microbiota of PF and AFmilk samples. A high relative abundance of *Rhodococcus fascians*, a species linked to vegetations,was observed in AF cow milk, while a high abundance of *Acinetobacter johnsonii* was detected inPF milk. The microbiota of Bitto cheese showed a prevalence of thermophilic LAB species and enterococci. Specifically, a high abundance of *S. thermophilus*, *Enterococcus faecium* and *Lactobacillus paracasei* was highlighted. This study showed that alpine pasture has a strong impact onthe microbial composition of cow milk, significantly increasing the abundance of some bacterial groups, specifically thermophilic LAB, recognized as natural starter culture, as also detected in Bitto cheese microbiota.

Influence of the cheesemaking process on the bioprotection activity of *Lactococcus* strains

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Nisin is a small peptide produced by *Lactococcus* strains that exerts an antimicrobial activity against pathogenic (i.e., *Listeria monocytogenes*) and spoilage bacteria (i.e., *Clostridium* responsible of blowing defects in cheese).
Five *Lactococcus* were selected for their ability to produce nisin at the optimal growth temperature (30 °C); one strain (FT27) was able to produce Nisin A, while the other 4 were Nisin Z producers.Moreover, *Lc. lactis* VC106 harboured, in addition to Nisin Z, the lct481 and lcnB genes coding for the Lacticin 481 and Lactococcin B synthesis.
At optimal growth condition, the bacteriocin production resulted to be strain dependent and a great variability was observed among the five biotypes (from 239 to 1995 IU/mL).
The strains were then used in preliminary trials to verify that they expressed their bioprotective activity in milk during the cheesemaking and ripening process that is typical of soft and semi cooked cheeses.
In this case, only one strain (VC106) was able to synthetize bacteriocins at cheesemaking temperatures, with maximum Nisin production of 729 IU/mL and persistence up to 170 days under refrigerated conditions.
The strain was then used in starter cultures in the Valtellina Casera PDO cheese productions. Twenty-one cheesemakings were performed in 4 different dairies plants, and in 13 out of 21 the antimicrobial activity was detected at different levels throughout ripening period, achieving the maximum after 110 days of storage.
This study provided important evidence on the bioprotection activity of *Lactococcus* strain VC106under real application conditions of semi-cooked cheese production.
For a fruitful use as bioprotective starter culture, further knowledge must be gained 1) about the interaction of the bioprotective strain with the starter lactic acid bacteria used in cheesemaking and 2) regarding process conditions that maximize bacteriocin production, as well as the kineticsof their degradation in the cheese during ripening.

Acknowledgements
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Dietary Microbes and Case Studies from Food Processing

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Dietary microbes can contribute in maintaining gut health and overall well-being, influencing digestion, immunity, and metabolic processes. The microbial content in food matrices is significantly affected by processing methods such as pasteurisation, cooking, and handling techniques, which can alter microbial diversity and load. Understanding and optimising factors that influence microbial content in foods can enhance the potential beneficial effects of dietary microbes, supporting the design of tailored investigations. This study explores a selection of case studies, interesting for the definition of microbe-depleted and microbe-rich diets. The impact of food processing on the microbial content of various food matrices, presenting case studies on dietary microbes in i) fermented food processing (i.e. pasteurisation of fermented table olives), ii) within relevant food categories (i.e. fruits, vegetables, cheeses), iii) associated with the fate of a given ingredient during different culinary reparations) (i.e. pecorino cheese). Using culture-dependent methods, we analysed interesting components of the microbial diversity/loads associated with the different matrices experimental matrices. These case studies underscore the critical role of food processing techniques in shaping the microbial loads associated to dietary components. Understanding these effects is crucial for optimizing food processing methods to enhance or preserve microbial content, improving food quality and potential health benefits. Our findings emphasize the need for furtherresearch to explore these dynamics across a broader range of foods and processing conditions. Bydoing so, we can develop more effective strategies in food science and nutrition to maintain or boost the live microbes in our diets.

Acknowledgements
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 within 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.

Creation of an “Egg-to-Meat” biobank of microbiota collected from broiler chickens raised with or without outdoor access.

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Chicken is the most consumed meat worldwide, with a variety of farming schemes from intensiveindoor to free-range systems which raises different concerns in terms of animal health and welfare. Recent studies suggest that microbial fluxes in chicken are influenced by the aviary environments, the diet, the broiler’s genotype and that various and successive microbial contamination or dispersion events occur in the production chain. A better understanding of these fluxes could help to identify potential levers for controlling microbiota and guaranteeing the health and robustness ofanimals, as well as the safety of the meat.
The objective of our project was to assess the effect of outdoor access of broiler chickens on bacterial fluxes along the production chain. Microbiota samples were collected from eggshells to carcasses as well as from aviary environment: 1) to analyse microbiota compositions using 16S rDNA ampliconsequencing and search impact of housing (indoors vs outdoor access) on chick colonisation, on the dynamics and shaping of the microbiota during breeding and on the composition of carcassesbacterial contamination; 2) to build a biobank of microbiota. Several zootechnical parameters have also been monitored.
This challenging and extensive study was made possible by the gathering of the varied expertise ofpartners in a INRAE consortium (France) involving two Poultry experimental units and five jointresearch units as well as a microbial Biological Resource Center dedicated to animal and human health (CIRM-BP).
A biobank of 620 samples was dispatched in a total of 1981 aliquots preserved at -80°C. The sampling points encompassed eggshells just after hatching, chick droppings and environmental samples during the whole breeding, ceca and carcasses of individualized chickens at slaughterhouse.
The biobank of samples is now available for further studies allowing reanalysis of certain samplesif necessary or new analyses such as a culturomics approach.

The work was done by the authors and the whole consortium Egg to Meat.

Lactiplantibacillus plantarum diversity and plant-based sources

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Lactic acid bacteria (LAB) play a crucial role in food systems due to their high potential to ensurefood quality, safety and shelf life. In particular, the species *Lactiplantibacillus* (*Lpb.*) *plantarum* emerges as a key model for the metabolic versatility, potential in the stress response and ecological diffusion, with a positive impact in food production where it is used as a starter culture, protechnological microbe, probiotic culture and biocontrol agent. Numerous strains of *Lpb. plantarum* havetheir genome sequenced and are available in specialized databases. There are also many studies on new selections of *Lpb. plantarum* from specific agro-food productions and from different ecological niches. Recently, the scientific community has turned its attention to plant-based matrices as unconventional reservoirs of new strains of *Lpb. plantarum* with distinct genetic and phenotypic traits, thus offering new opportunities to design bio-based innovation in the food industry. Here, we present an overview of the ecological origin of different *Lpb. plantarum* strains, both completesequenced strains and strains from new selection. In mapping the different origins associated with the intraspecific diversity of *Lbp. plantarum*, we report some new sources of isolation we recently performed, linked to agro-food and the world of plants, highlighting the future efforts that are necessary to increase the ecological representativeness of this species in dedicated bio-banks.

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Lactiplantibacillus plantarum and bio-based solutions in food biotechnologies

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Lactic acid bacteria (LAB) play a crucial role in food processing, as they can positively affect safety and all the aspects of food quality (e.g. sensory, nutritional, functional), and they also haveinteresting applications in agriculture. LAB represent bio-resource to design solutions inspired by nature to support the green transition of agro-food systems. Among LAB, lactobacilli are a heterogeneous group of different genera (recently re-classified from the taxonomic point of view) with relevant applications from the farm to the fork. Here, we propose the species *Lactiplantibacillus* (*Lpb.*) *plantarum* as a model species for lactobacilli because of the nomadic lifestyle and the huge versatility in ecological diffusion. In contrast to many other lactobacilli species, which have a spectrum of adaptation to a limited number of ecological niches, the ubiquity of *Lpb.plantarum* emphasizes the wide range of metabolic targets and the potential in the stress response. *Lpb. plantarum* strains are used as starter cultures, probiotic cultures, and biopreservation agents, with several properties that are used to pursue economic, social, and environmental sustainability.Through a selection of case studies, we want to provide an overview of the broad-spectrum potential of *Lpb. plantarum* in agro-food productions, with a particular focus on the protechnological, probiotic and biocontrol traits that configure this species as an example for bio-based innovationsin the field of food biotechnology.

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Enhancing bioprotection on fresh-cut apples with microwave heating: preliminary tests for inclusion in hurdle technology

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The inclusion of microwave (MW) and biocontrol agents in a 'hurdle' approach represents a novelmethod for preserving the quality and safety of fresh-cut produce. This study explores the application of MW heating to enhance the bioprotective efficacy of *Lactiplantibacillus plantarum*, employing a Central Composite Design (CCD) and response surface methodology to determine the optimal treatment conditions for minimally processed apples. Microwave treatment parameters, including power level and treatment time, were varied according to the CCD matrix. The effect of MW applied before or after *L. plantarum* (UFG 121) inoculation, aiming to identify the conditions that can enhance the biocontrol efficacy of this strain without compromising the apples quality was studied. Preliminary results demonstrated that the power level and exposure time influenced the extent of the MW impact. Optimal conditions identified through CCD analysis indicated a synergistic effect between moderate MW power and shorter exposure times, which enhanced the survival of the biocontrol agent. Quality parameters, including colour, visual quality, and chemical aspects, were assessed post-treatment and after 7 days of storage. The optimal MW treatment should maintain the apples colour while minimizing quality losses. The sensory evaluation confirmed that fresh-like attributes were significantly affected by power and time of treatment. In conclusion, the study established that a carefully calibrated microwave treatment, in conjunction with the application of lactic acid bacteria, could be a suitable technology to extend the shelf life and safety of fresh-cut apples. The CCD approach was exploited to preliminarilydefine the optimal conditions, balancing microbial survival and product quality. This dual treatment strategy holds promise for the fresh produce industry, offering a feasible method to deliver high-quality, fresh, and safe products to consumers.

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Biocontrol, edible packaging, and physical technologies: towards the design of integrated solutions for postharvest fruits and vegetable management

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The application of hurdle technology and integrated solutions in postharvest handling integrates multiple conventional and emerging preservation methods to ensure microbial safety and maintain the quality of minimally processed horticultural products. This approach, tailored to specificmatrices and challenges, exploits the synergistic effects of various agents, which are not individually decisive, to create a series of preservative factors that microorganisms cannot overcome. The primary hurdles in food preservation include high or low temperatures, natural or synthetic preservatives, biocontrol microorganisms, packaging solutions and emerging mild physical treatments. In the context of fresh fruit and vegetable minimal processing, the main objective is to meet safetystandards without compromising food quality and consumer health. Innovative combinations of hurdles, their intensity, and their sequence offer a promising future for minimally processed fruits and vegetables, reducing the intensity of single techniques while preserving freshness and quality.This work focuses on the main hurdles/integrated approaches that include at least one emerging physical treatment and/or one edible packaging solution and/or one biocontrol application, assessing their impact on the quality and safety of fresh-cut fruits and vegetables. Combining emerging physical approaches or edible packaging solutions with microbial biocontrol agents represents a promising area of study. Applying physical treatments in combination with biocontrol inoculationcould enhance the effectiveness and dominance of biocontrol agents, offering a dual mechanism for improving the safety and quality of fresh produce. The inclusion of biocontrol agents in edible packaging presents new opportunities for the application of lactic bacteria on the surface of products, allowing their contribution to be enhanced with respect to the overall quality of the products.

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Application of lactic acid bacteria in the postharvest of fruits and vegetables

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The heterogeneous group of lactic acid bacteria (LAB) provides the basis of several bio-based solutions in postharvest applications. The complex microbial ecosystems associated with fruit and vegetables include a wide variety of naturally associated bacteria, yeasts, and moulds. This microbiota may be connected with undesired pathogens, microbes displaying spoilage activities, but can also represents the source of new LAB isolations. One of the main sustainable applications of LAB on fruits and vegetables concerns the potential for biocontrol. After harvest, the handlings may increase these undesired microorganisms, negatively impacting the quality of these products andcausing a reduction in yield, shelf-life, safety and marketability, with consequent production losses. Biocontrol, considered one of the most sustainable postharvest approaches, consists of applying selected microorganisms that can limit the development of undesired ones. Selected LAB have been successfully applied on several types of fresh products (leafy green, mixed salads, lettuce, potato, mushroom, tomato, melon, apple, table grape, strawberry, kiwifruit, banana) having as target microorganisms mainly pathogenic bacteria (e.g., Listeria monocytogenes, Escherichia coli) and/or moulds (e.g., *Botrytis cinerea*, *Penicillium expansum*). LAB not only improve shelf-life and safety, but can also contribute to enhancing the nutritional and functional quality of foods, enriching products with probiotics and dietary microbes, and supporting the beneficial aspects associated with this type of matrix. The combination of LAB with other physical, chemical and biological strategies to obtain synergistic effects is a much-discussed aspect, highlighting the importance of studying the stress response in bacteria as a driver for the design of innovative solutions.

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Microbial Diversity from fermented Apulian '*Gentile di Puglia*'sheep milk: polyphasic characterization, dairy valorisation and cross-over cereal based applications

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Traditional fermented dairy products are important for preserving Geographical Indications and gastronomic heritage. These foods and beverages are also source of nutrients and of functional properties and a reservoir of lactic acid bacteria interesting for the design of new starter cultures and biotechnologies. This study aimed to perform a polyphasic characterisation of the microbial diversity of fermented '*Gentile di Puglia*' sheep milk from Subappennino Dauno (Apulia, Italy). Sheep milk samples were fermented under different conditions to selectively isolate microorganisms. Gram staining, catalase, CO_2 production analysis, and microscopic evaluation were performed to perform a preliminary characterisation of the isolates. Additionally, the antimicrobial and antifungal activities of all the isolates against several targets representative of spoilage and pathogens in the food industry were also assessed. The study identified a diverse microbial population in the fermented sheep milk, with the heterogeneous group of lactic acid bacteria (LAB) predominating. Protechnological and genetic characterisations have been performed on a panel of strains. Based on the results, some selected LAB strains were used as a multi-strain starter culture to produce Pecorino cheese from '*Gentile di Puglia*' sheep milk. The application of selected strains in Pecorino cheese production has been performed to obtain a different sensory profile and to improve shelf-life compared to commercial starter culture, highlighting the importance of valorising local dairy products by exploiting autochthonous microbiota. Finally, a selection of LAB strains have been tested in cereal matrices to evalutate the design of plant-based yoghurt-like products. The cereal tested in the trial were also original of Daunia region, promoting an experimental plan thatvalorises animal, plant and microbial biodiversity associated with a specific marginal area.

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Characterization of the rib operon riboswitch in riboflavin overproducing *Lactiplantibacillus plantarum* strains

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Lactic acid bacteria (LAB) are able to produce functional and bioactive compounds for the human health, such as B vitamins. In LAB, the expression of the rib operon for the synthesis of riboflavin(vitamin B2) is regulated by an FMN-riboswitch consisting of a sensitive domain (aptamer) that binds the effector (FMN) and induces a conformational change in the regulatory domain. In this work, LAB strains isolated from wild fruits of the Mediterranean area were tested for their prototrophy for riboflavin in a chemically defined riboflavin-free medium (CDMRF). The strains able to survive in the absence of riboflavin were then exposed to roseoflavin (a toxic analog of riboflavin). This treatment allowed the selection of spontaneous mutants that had mutations in the riboswitch of the rib operon and were capable of overproducing riboflavin. The ability to produce vitamin B2 in the CDMRF medium was evaluated by fluorescence. *L. plantarum* strain 187 was found to produce the highest amount of riboflavin (between 5 and 6 mg/L). Molecular characterization of the riboswitch variants was performed for all previously selected mutants by aligning the sequence of the regulatory element of the mutants with the parental. In particular, we observed that the highest vitamin B2 producer L. plantarum 187 had a G to T mutation at position 91 (relative to the transcription initiation site), while the lowest producer had a C to A mutation at position 78. Work is underway with the best vitamin B2-overproducing *L. plantarum* strain and its ability to produce functional foods, such as fermented plant-based beverages, will beevaluated using different substrates and growth conditions.

Bioprospecting and valorization of citrus crops to obtain preharvest and post-harvest biocontrol agents.

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Introduction: Citrus fruits are among the most widely grown fruits globally. In 2022, the Food andAgriculture Organization estimated their production at 158.5 million tons, with about one-third of the produce lost to fungal rot. In addition, 20-30% of the inedible parts of these fruits generatesignificant food waste.
Objectives: To investigate the microbiome of citrus fruits and crops and select and characterize microbes for developing natural alternatives through microbial fermentations using citrus residues. The aim is to prevent fungal contamination and reduce losses during field and storage.
Methods and Results: To isolate strains of lactic acid bacteria (LAB) and fungi from different citruscrops. An initial test determined the antifungal activity of the LAB strains. Selected strains were used to ferment a medium with citrus residues. Antifungal metabolites were studied, and *in vitro* tests were performed against pathogenic fungi isolated. The most effective treatment was applied as a biocoating on fruits to test its biopreservation capacity. The metabolome of the fermented medium was studied to determine its biostimulant and biocontrol capacity, aiming to develop dual purpose products that enhance citrus crop productivity and prevent pathogenic fungal infections.Strains N3B1 showed the best antifungal activity, with MIC/MFC values between 1.8-250g/L. These culture media had a presence of antifungal metabolites, such as lactic, acetic, DL-3-phenyllactic, 3-4-dihydroxyhydrocinnamic, salicylic, and vanillic acids. Tests on oranges showed that treatment reduced the proportion of contaminated oranges by 90% after 10 days of storage and decreased the presence of fungi by 4Log10 units of spores/g of citrus.
Conclusions: The results demonstrate the potential of the fermented medium with citrus residuesas a biostimulant and biocontrol agent for the development of natural products in the field and storage, exemplifying a circular economy model.

Fermentation as a driver of innovation for the development of the agri-food sector in marginal areas

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The social, economic, and environmental management of marginal areas is crucial for sustainable development and resource valorisation in Italy. Preserving and enhancing biodiversity is a key strategy to promote the innovation of agro-food systems in these regions. Improving the quality and safety of fermented products, selecting indigenous microorganisms, studying fermentative microbiomes, and promoting microbial biotechnologies are important factors supporting green transition in the food sector of marginal areas. In this context, we present an overview of significant advances at the international scale in the field of microbial management to generate addedvalue and support new product development in the food sector in marginal areas. In addition, we propose the Apulian region (Italy) as a model territory for studying this type of valorisation. By mapping products and resources that represent good targets for regional development, we aim to create added value through fermentation and the exploitation of microbial diversity. The mappingincludes a review of geographical indications, traditional, typical and artisanal products, and rawmaterials that can be processed through fermentation or using microbial-based solutions.

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Innovation in food fermentations and the cross-over fermentation concept in new product development

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Fermented products represent a dynamic and constantly evolving sector, that can offer solutionsto improve the well-being and nutrition of different population targets. Fermentation also makes it possible to promote sustainable innovation and enhance the quality of foods and beverages. Among the various dynamics of innovation in this sector, the concept of 'cross-over fermentation' in the development of new products should be underlined. Cross-over fermentation has been defined as "processes in which a microorganism from one traditional fermentation process is introduced onto a new substrate and/or to a new partner" [1]. Here, we report a series of case studies to highlight the potential of cross-over strategies in valorising the microbial biodiversity associated with traditional fermentations to design novel fermented foods and beverages. It is a trend that allows the exploitation of the latent biotechnological potential related to studies of isolation and characterisation of autochthonous bacteria, yeasts and filamentous fungi from spontaneous fermentations. In addition, this approach makes it possible to improve the added value ofraw materials and stimulate the development of new products, creating a link between traditionalfermented products and innovative fermented products. All these aspects are particularly interesting to promote the sustainable potential associated with fermentation processes and to supportthe development of a plant-based sector designed with solutions inspired by nature and not with ultra-processed foods.

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Selection of *Bacillus strains* as potential candidate against pathogenic *Fusarium* occurring on cereal

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Fusarium species are among the most important pathogens of cereals able to synthetize harmfulmycotoxins. The most important cereal diseases are caused by co-occurrence of multiple *Fusarium* species, mainly *F. graminearum* and *F. culmorum*, producers of deoxynivalenol, and *F. proliferatum*, producer of fumonisins. Nowadays, the increasing interest to reduce chemicals in agriculture, prompts researchers to select eco-friendly strategies against fungal diseases.
This study aimed to select potential bacterial bio-control agents to control *Fusarium* species. Forty-eight *Bacillus* strains belonging to *Bacillus velezensis*, *B. amiloliquefaciens*, *B. subtilis*, *B. licheniformis*, *B. mojavensis*, *B. simplex*, *B. megaterium*, *B. oleronius*, *B. pumilus* and *B. safensis*, isolated from wheat and maize kernels, were tested. The antagonistic activity against *F. graminearum*, *F. culmorum* and *F. proliferatum*, by co-colture assay, and the antimicrobial effect of bacteria filtrates were evaluated. Twenty-three strains, including all *B. velezensis* and *B. amyloliquefaciens* strains, showed a good antagonistic activity, with mycelial growth inhibition values up to 70%. Moreover, 14 strains produced active compounds inhibiting mycelial growth up to 60%. To elucidate the molecular mechanisms associated to the activity of these strains against *Fusarium* species, all selected strains were screened for the presence of genes involved in the synthesis of active biomolecules, including surfactin, fengycin, iturin, bacillomycin, bacilysin, difficidin and mycosubtilin. The gene fragments were also sequenced and analysed, to identify, among and within *Bacillus* species, possible polymorphisms associated to the variable capabilityto inhibit *Fusarium* growth. In addition, the possible capability of *Bacillus* strains to influence deoxynivalenol and fumonisin production was studied. Although no activity was observed againstfumonisin production, half of *Bacillus* strains inhibited completely DON production.

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Effect of plant essential oils on *Botrytis cinerea*

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Botrytis cinerea is a fungus that causes pre-harvest and post-harvest diseases of various plants and cabbage (*Brassica oleracea* convar. *capitata*) is one of them. *B. cinerea* causes grey mould of cabbage and this disease is little significant in the pre-harvest phase, but is a serious problem forstored cabbage heads. Protecting stored cabbage is difficult because the use of synthetic chemicalplant protection products (pesticides) is very limited to protect the health of consumers during storage. Some essential oils have the ability to reduce the growth of certain fungi and are environmentally friendly. The ability of thyme, cinnamon, cloves, oregano and lemongrass essential oils to suppress the growth of *B. cinerea* strains from Culture collection of microorganisms of Crop Research Institute (VURV) was investigated in *in vitro* experiments. Essential oils at five concentrations were added to agar culture medium in Petri dishes and their effect on the growth of *B. cinerea* colonies was evaluated. Differences were found in the effect of individual essential oils and between different concentrations, as well as between different strains of *B. cinerea*. Oregano essential oil inhibited growth the most, not allowing growth even at a concentration of 83.33 l/l. Another essential oil that significantly inhibited the growth of *B. cinerea* colonies (completely at the highest concentration evaluated, 250 l/l.) was thyme essential oil. Cinnamon and clove essential oils reduced the growth of *B. cinerea* colonies. The effect on colony growth in all strains examined was evident at all concentrations for these two essential oils, especially at the highest concentration evaluated, 250 l/l. Lemongrass essential oil reduced the growth of *B. cinerea* strainsless, but still suppressed colony growth at the highest concentration evaluated (250 l/l). At lower concentrations, growth was less restricted.

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Lactic acid bacteria as microbial starters in malting by-product fermentation to obtain innovative bread formulation

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Sourdoungh-based preparations represent attractive models for fortifying food product with microbially treated by-products. The malting by-products represent a significant waste and they have not been exploited in details. Malting rootlets and malt glumellae were selected to test fermentation by LAB previously characterized for other food applications as microbial starters, with the final aim to include the fermented by-product in one innovative bread formulation to increaseshelf-life, digestibility and nutritional value.
Malting by-products have been characterised to study the presence of microbial contaminants before and after the fermentation process. In detail, *Lactiplantibacillus plantarum* (PLA) and *Lacticaseibacillus rhamnosus* (RHM), isolated from milk and cheese respectively, were individually inoculated at initial cell density of ca. Log 7.0 cfu/g in malting rootlets and mixture of rootlets and glumellae dough (dough yield of 400). The dough was fermented at 30 °C for 24 h, and the growth and acidification of dough fermented by PLA and RHM were monitored every 2 hours. At each sampling hour, LAB count, pH and titratable acidity of the dough were measured, while dough colour, antioxidant activity and total phenols were detected at the beginning and the end of fermentation.
The enterobacteria in malting rootlets and malting rootlets and glumellae decreased at a densitylower than 100 CFU/g. The total free amino acids of doughts fermented increased up to 140%. The growth and acidification of dough fermented by PLA and RHM showed a lag phase lower than3 hours for both parameters. High growth speed was found in rootlets, while higher acidificationspeed in mixed matrix.
Finally, preliminary results on the inclusion of fermented and unfermented malting rootlets (5% w/w of flour total weight) in fortified bread formulations showed good sensorial acceptability.
The inclusion in the sourdough of two different strains of yeast will be tested.

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Unconventional raw plant matrices as source of pro-technological microbes for application in food fermentation.

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The use of unconventional raw materials within food processing is becoming a widely used sustainable and innovative approach for developing food products with enhanced nutritional, organoleptic, technological, functional and preservative characteristics to satisfy the increasing request for a healthy diet. Alternative matrices can also be a source of microbial strains with enzymatic features, which can be useful for enriching food ingredients in bioactive compounds. Biotechnological processes of fermentation have been used for centuries to produce food often using selected starters, such as lactic acid bacteria (LAB) and yeasts with enhanced nutritional, organoleptic, technological, functional, and preservative molecules. However, the selection of a good candidate as starter strain needs molecular characterization and evaluation of safety and potential pro-technological activities.

In this work different cereal by-products (flours of wheat bran and wheat germ, defatted or not), vegetable by-product (fresh substandard peas) and finally flours obtained from Okra (*Abelmoschus esculentus* L.), a native African plant, were collected.

In order to isolate beneficial microorganisms, these by-product matrices were plated onto MRS agar. A total of 20% of the colonies randomly picked from countable plates, were purified and stored at -80°C. The bacterial isolates were characterized by repetitive extragenic palindromic PCR (REP-PCR) and isolates representative of each REP-PCR profile (19) were identified by sequencing of the 16S rRNA gene. Among strains isolated, only those belonging to the Qualified Presumption of Safety (QPS) list were characterized for antibiotic resistance, biogenic amine production, EPS production and proteolytic activity.

Bacterial strains were deposited in the CNR-ISPA Culture Collection (ITEM Culture Collection) in cooperation with SUS-MIRRI.IT (an Infrastructure Research Project of the National Recovery and Resilience Plan (NRPP).

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Real-Time investigation of *Lactiplantibacillus plantarum* Fermentation in Experimental and Real Food Conditions Using a Metabolomics Approach.

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Many food industries use *Lactiplantibacillus plantarum*, a ubiquitous facultative heterofermentative lactic acid bacterium (LAB), as a starter culture due to its technological potential and versatility in adapting to different food environments. To understand and optimize fermentation, 'omics'technologies, especially metabolomics, are employed, offering insights into the metabolic state ofmicroorganisms and the dynamics of metabolic pathways. Microbial volatile organic compounds (VOCs), which influence odour and flavour, serve as biomarkers to track microbial metabolism during fermentation, providing information on the quality of the matrices. Among the sensors used to monitor VOCs in the food and beverage sector, Direct Injection Mass Spectrometric (DIMS) techniques have gained increasing interest due to their versatility, real-time analysis, and strong analytical performance. Proton-Transfer-Reaction coupled with Time-of-Flight Mass Spectrometer (PTR-ToF-MS) is a prime example of DIMS technology, known for its sensitivity, accuracy, time efficiency, non-invasiveness, and eco-friendly analysis. PTR-ToF-MS measurements allow for the assessment and monitoring of VOCs i) during fermentation processes to track their evolution andpotential reaction kinetics, and ii) in final products to provide information on possible consumer sensory experiences and product quality. This technique offers a green alternative for profiling volatilome during food fermentations [1]. In this study, PTR-ToF-MS was used for the online monitoring of the fermentation of i) six *L. plantarum* strains in MRS for 32 hours, and ii) one selected *L. plantarum* strain from the previous experiment in three plant-based (soy, almond, andoat) beverages and one animal-origin beverage (milk) as a control for 72 hours. This preliminaryinvestigation also aims to develop a PTR-based strategy to build a reference framework for the development and design of fermented beverages.

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Cell surface proteome of *L. plantarum* strains isolated from vegetable foods and dairy products

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Lactiplantibacillus plantarum (formerly *Lactobacillus plantarum*) exhibits relevant probiotic andtechnological features and is widely used in agrifood sector as plant growth-promoting bacteria and to improve quality properties of food products. Surface proteins are directly involved in functional and technological traits of bacteria and their dynamic cross-talk with the host. Many genomic studies were carried out to predict the exoproteome of this species revealing the presence of several classes of proteins differently linked to cell surface [1]. Although proteomics deeply contributed to investigate surface architecture of bacteria cells leading to define protein location and topology [2], a limited number of proteomic studies have investigated the repertoire of *L. plantarum* surface proteins. In this context, we performed a shotgun proteomic study to identify the proteins present on the surface of four different *L. plantarum* strains (two isolated from vegetable foods, V strains, and two from dairy products, D strains) aimed at highlighting differences in protein profiles potentially related to habitat of origin and specific properties of the analyzed strains. Proteins predicted to contain a signal peptide for extracellular translocation, transmembrane proteins and moonlighting proteins known to be involved in adhesion processes were identified. Results showed a more complex pattern of surface proteins in V strains compared to that from D strains. Cell surface proteomes of V strains were characterized by a higher number of proteins displaying glycosyltransferase activity and peptidoglycan hydrolases compared to those of D strains. Peculiarmolecular functions of proteins identified in V strains suggested a probiotic and biotechnologicalpotential of these bacteria.

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Biodiversity in natural fungal community isolated from cheese aged in Apulian cave

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Filamentous fungi contribute to the organoleptic characteristics, texture and color of long-ripened cheese, with their metabolism, acting as secondary starters.
Traditionally worldwide cheesemakers gathered empirically that cheese production is affected by environment, technological procedures, and especially by microorganisms. Cheesemakers have naturally selected through time strains able to ensure the correct seasoning of cheeses, from which later some strains became commercial cultures, intentionally inoculated for driven-maturations of cheese.
Currently, there is a reversal trend compared the past that appreciates traditional protocols for food manufacturing in light of an increased awareness of the importance of food safety. Therefore, the interest in typical local foods and traditional protocols have led to branch out the way of aging, exploiting sustainable ripening in natural environments like caves, similarly to the past. In these natural environments, cheeses come into contact with non-dairy microorganisms, potentially able to affect positively or negatively cheese aging.
The mycobiota of caciocavallo cheese, aged in cave of an Apulian dairy factory, was analyzed to select pro-technological molds compatible with cheese aging, according to quality and safety parameters. Fungal community was isolated, taxonomically identified by DNA-based analysis and characterized for their relevant technological properties. Two strains belonging to non-toxigenic species, *Penicillium flavigenum* and *P. biforme*, were selected as secondary starters for caciocavallo pilot aging in cave. Main results related to their isolation, characterization, inoculation of cheese and the preliminary sensory analysis are here reported.

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Dried cell-free supernatant of *Bacillus subtilis* strain ET-1against *Penicillium digitatum* on clementine fruits

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In this work a low medium for *Bacillus subtilis* growth was designed with the aim to obtain a cell-free supernatant (CFS) rich in secondary metabolites for post-harvest control of green mould. For medium development, culture growth trials with *B. subtilis* ET-1 strain were carried out in flasks using different agro-industrial wastes as carbon and nitrogen sources and Iturin A concentration was determined. The medium with higher level of Iturin A (2,45 g L-1) was choice and used for scaling up process. In specific, the bacterium was grown in a 21 L stirred-tank bioreactor with working volume of 10 L at temperature of 25 °C and without pH controlling.
At the end of culture, the supernatant was clarified from the cells by centrifugation and five litres of CFS was stabilized by drying processes with skim milk addiction.
Then, the effectiveness of both liquid and stabilized CFS against *Penicillium digitatum* on citrus fruits (*C.* × *clementina*) in vivo was evaluated. For the experiments, sanified fruits were injured and treated before the artificial inoculation with 20 µL of *P. digitatum* suspension at concentration of 2,3x104 conidia mL-1.
The measurements were carried out when the first symptoms of the disease appeared and concerned the presence of infection on the individual wounds.
The results of the artificial inoculations show the excellent disease control effect on the fruits protected with CFS of the ET-1 isolate.
Furthermore, no significant difference in the protection effect was observed between the theses treated with liquid and stabilized CFS. Therefore, the stabilization process conducted with drying technology did not reduce the antifungal activity of the secondary metabolites present in CFS.
In conclusion, the ET-1 isolate could be usefully used to produce alternative formulations to conventional chemical fungicide, thus achieving the objective of the EU "farm to fork" strategy aimed to produce healthier and more sustainable food.

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Penicillium nalgiovense: a promising fungal starter to control *Aspergillus westerdijkiae* contamination in salami

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Aspergillus westerdijkiae is a cause of concern in salami production in warmer climates since it can grow on the product surface and synthesize ochratoxin A. This study aimed to evaluate the capability of *Penicillium nalgiovense*, a common mould starter used for salami production, to control the growth of *A. westerdijkiae* during salami production Salami were co-inoculated with *P. nalgiovense* and *A. westerdijkiae* with contamination rates up to 32% of the spoiler, besides a control with each single species, and a respective salami only inoculated with *A. westerdijkiae*. Salami were seasoned in maturation chambers with controlled temperatures and relative humidities. Mycological analysis has been conducted at the end of seasoning by spread plating on 18% Dichloran Glycerol Agar. Generally, *A. westerdijkiae* colonies were visually not observed in the salami co-inoculated with *P. nalgiovense*, independently of the inoculation rate accessed. On the other hand, *A. westerdijkiae* was able to colonise the salami when no *P. nalgiovense* was co-inoculated. Results demonstrated that the biological control activity of *P. nalgiovense* as starter can limit the *A. westerdijkiae* growth even under high rates of contamination, preventing the human exposure to ochratoxin A.

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Biodiversity in fungal community on cheese aged in Dossena mine

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Accurate identification of the fungal community spontaneously colonizing food products, aged in natural and not controlled environments, provides information about potential mycotoxin risk associated with its consumption.
Autochthonous mycobiota colonizing cheese aging in Dossena mines, was investigated and characterized by two approaches: microbial isolations and metabarcoding. Microbial isolations and metabarcoding analysis were conducted on cheese samples, obtained by four batches, produced in four different seasons of the year, aged for 90 and 180 days, by five dairy farms. The two approaches, with different taxonomical resolution power, highlighted *Penicillium biforme* among filamentous fungi, collected from 58 out of 68 cheeses, and *Debaryomyces hansenii* among yeasts, as the most abundant species (31÷65%), none representing a health risk for human cheese consumption. Shannon index showed that the richness of mycobiota increases after 180 days of maturation. Beta diversity analysis highlighted significant differences in composition of mycobiota of cheese produced by different dairy farms and aged for different durations. Weak negative growth interaction between *P. biforme* and *Aspergillus westerdijkiae* by in vitro analysis was observed leading to hypothesize that a reciprocal control is possible, also affected by natural environmental conditions, possibly disadvantageous for the last species.

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Proteolysis in soy tempeh obtained with commercial starter

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Tempeh is produced from soya or other legumes fermented by beneficial microorganisms such as moulds and lactic acid bacteria (LAB) that contribute to modify the nutritional composition of the food matrix, by improving the bioavailability of nutrients and phytochemicals [1]. The microbial fermentation process also produces biopeptides with biological effects, which are of great interest in the context of functional foods [2, 3]. The aim of the present research was to study the proteolytic activity of a commercial mold starter during the production of soy tempeh and the microbiota composition of the commercial starter and the final fermented tempeh product.
The biological soybean was soaked for 12 h, boiled for 1.5 h, drained, inoculated with commercial starter mould and incubated in perforated plastic bags at 30°C for 48 h. At time zero, after boiling and after 48 h fermentation, pH, microbial count, degree of protein hydrolysis, bioactive peptides were evaluated. Furthermore, microbiota identification of the commercial starter culture as well as the final tempeh products was evaluated by metabarcoding analysis of 16S rRNA for the identification of bacteria and ITS2 for that of fungi. The proteolytic events occurring during the fermentation process were evaluated by gel-based and gel-free proteomics analysis.
Tempeh starter was composed mainly of a pure culture of *Rhizopus microsporus*. The microbiota of the final tempeh products was dominated by the most abundant genera also detected in the commercial starter, although other bacterial and fungal genera were identified, probably related to environmental contamination.
Proteomics profiling were characterized, identifying several hundreds of peptides released mainly from glycinin and conglycinin soybean proteins.

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Relationship between the chemical content of fruits and endophytic fungal communities in black elder *Sambucus nigra* L.

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It has previously been shown that endophytic microbial communities can affect the phytochemical content of medicinal plants. Here we investigate whether the phytochemical content of wild elderberries is related to endophytic fungal communities inhabiting wild elder plants *Sambucus nigra* L.
Fruit samples were collected at three different stages of maturity from wild *S.nigra* populations located in Latvia. Total DNA was isolated from surface-sterilized fruits and endophytic fungal communities were characterized using ITS1 amplicon NGS sequencing. The phytochemical content of ripe fruits was characterized by assessing total flavonoid content and the content of five dominant compounds was determined using HPLC.
Endophytic microbial communities of *S.nigra* fruits were significantly affected by the geographical location of the sampled population and by the content of one of the dominant chemical compounds of fruits. The diversity and richness of endophytic fungal communities decreased with the progression of fruit ripening. Although the chemical composition and fungal communities significantly varied among studied populations we did not observe the correlation between fruit chemical content and endophytic fungal communities (Mantel r=0.01; p=0.407). Further studies assessing the effect of individual fungal taxa on fruit phytochemistry might reveal more intricate relationships between endophytic fungal communities and the phytochemical content of *S.nigra* fruits in the future.

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Genomic characterization of *Companilactobacillus* spp.: assessing safety and application potential in the food chain

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Companilactobacillus crustorum is a lactic acid bacterium (LAB) isolated from Belgian wheat sourdough [1], but also found in dairy products and forages [2]. Members of *Companilactobacillus* genus, formed by 34 species, occur consistently in food fermentations of plant and animal origin [2].
Despite the possible applications in food and feed, only two *Companilactobacillus* species hold the Qualified Presumption of Safety (QPS) status, as defined by the European Food Safety Authority (EFSA), therefore a thorough safety assessment is crucial for determining applicability. In this perspective, the availability of over 100 *Companilactobacillus* spp. genome sequences allows the investigation of possible interesting traits related to safety and application.
This study investigates the genomic landscape of *C. crustorum* and related species, focusing on antibiotic resistance genes (ARGs), sanitizing agents resistance genes (SARGs), antimicrobial peptide sequences (AMPs), and biosynthetic gene clusters (BGCs), with an emphasis on their potential implications for food safety throughout the farm-to-fork continuum.
High-quality genomes of *Companilactobacillus* spp. were downloaded from the NCBI database. The nf-core/funcscan pipeline [3] was utilized to predict and annotate ARGs, SARGs, AMPs, and BGCs. In silico analysis offers insights into the distribution of ARGs and SARGs across the genus, aiding in the differentiation between acquired and intrinsic resistance.
The presence of AMPs and BGCs highlights the potential of these bacteria for various applications, while also raising concerns about their impact on food safety and microbial ecosystems along the food chain. The production of AMPs and BGCs could influence microbial communities in food products, affecting spoilage, fermentation processes and, potentially, consumer health. This study contributes to safety assessment of *C. crustorum* and related species within the context of the EFSA QPS approach.

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Screening of yeast and filamentous fungi from VTT Culture Collection for cacao butter and palm oil production

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Yeast and filamentous fungi strains obtained from VTT Culture Collection were screened for theirlipid profiles. The objective was to find strains with natural ability to produce fatty acid profiles similar to palm oil or cacao butter. VTT's culture collection contains more than 3000 yeast and filamentous fungi strains, of which 27 yeast strains belonging to 18 different species and 16 filamentous fungi strains belonging to 7 different species were selected based on a literature search revealing the most potential candidates.

Candidates were grown on C/N 75 and PD media and their growth, total lipid content and fatty acid profiles analyzed. The total lipid content varied from 5% to 42% (yeasts) and from 8 to29% (filamentous fungi). Fatty acid profiles varied significantly among yeasts, especially share ofpalmitic acid, oleic acid and stearic acid, which are the main constituents of cocoa butter and palmoil.

Three different industrial side streams, obtained as by-products of different food processes, were used a substrates to cultivate the best candidates from the preliminary screen. Two of the side streams were lactose rich whereas one contained mainly glucose and galactose.

For cacao butter and palm oil production, the best candidates were found among yeast strains. Two of the yeast strains produced fatty acid profile close to cacao butter and one yeast strain closeto palm oil. None of the fatty acid profiles of filamentous fungi screened resembled cocoa butter orpalm oil. Instead, fatty acid profiles of some filamentous fungi were very close to meat fatty acid profile.

Diversity of Non-Conventional Yeasts (NCY) for innovating fermented beverages: investigating microbiomes

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Fermented food and beverages represent an important part of the planetary cultural heritage, where microbial consortia contribute to the broad range of sensory and functional properties. Yeast biodiversity plays a crucial role for product diversification in fermented beverages (FB) [1]. Interestingly, fermenting abilities are present not only in Ascomycota, that includes the fermentation workhorse *Saccharomyces cerevisiae*, but also in the much less explored phylum Basidiomycota, with the interesting example of Mrakia, with no history of use in the food chain, but with a high innovation potential [2].

In the framework of a research project focused on nonconventional yeasts for FB, an updated version of FoodMicrobionet (FMBN) was realized: FMBN is a food-dedicated and curated repository of metabarcoding studies [3] and it now includes fungal sequences [4], from 1114 samples described in 21 published studies, including, at the date of writing of this abstract (June 2024) 3 on wine, 1 on beer and 6 on other alcoholic beverages.

FMBN 5.0 was used with the two-fold aim of evaluating possible presence of Mrakia-related sequences in analyzed datasets, and to define the most important genera that could deserve attention for future studies.

As for Mrakia-related sequences, as expected, they were detected at very low levels (relative abundance < 0.25%) in FMBN FB datasets. For this reason, an in-depth characterization of the species is necessary to assess their safety for application in fermentation, before any intentional use in the food chain can be authorized.

As for other genera, the most important ones in terms of prevalence and median abundance in alcoholic beverages were *Saccharomyces*, *Hanseniaspora*, *Pichia*, *Cladosporium*, *Candida* and *Aureobasidium*.

Data presented here on fermented beverages shows how digitalized data on microbiomes are powerful tools to generate new knowledge and insights for developing bioeconomy, supporting responsible innovation in food systems.

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Principles for selection of strains of microorganism cultures for the creation of biopreparations of agricultural designation

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Nowadays it is definite that the global growth in the production of biological/microbiological fertilizers is due to the partial, and in some cases, complete replacement of mineral fertilizers in agricultural technology.

The main reason for changes in approaches to intensive agriculture is the ineffective absorption by plants of nitrogen, phosphorus and potassium in the composition of agrochemicals, leading tothe gradual salinization of cultivated lands. At the same time, the increase in consumption of agricultural products of plant origin necessitates expanding the range of biological preparations with improved properties for more efficient farming, including organic agriculture. In this regard, the crucial importance lies in the targeted search for new types of microorganisms endowed withincreased stress resistance and adaptive capabilities in various types of soils; valuable metabolic characteristics; a powerful enzymatic system; high nitrogen-fixing and phosphate solubilizing ability; antimicrobial, antifungal, selective insecticidal activity; and resistance to various pesticides (pesticides, herbicides, fungicides) when used together; long shelf life and viability.

When selecting promising cultures of microorganisms, special attention should be paid to the reduction of cost of production of biopreparations, the safety of their use in relation to the environment, humans, animals, birds and, especially beneficial insects.

Thus, the goal of our research is to develop the technological basis for the production of biopreparations of agricultural designation using active strains of bacteria *Azotobacter chroococcum*, *Paenibacillus polymyxa*, *Paenibacillus mucilaginosus*, *Priestia megaterium*, *Rhisobium*, *Pseudomonas chlororaphis*, *Bacillus thuringiensis*, *Trichoderma viride*, *Metarhizium anisopliae*, *Beauveria bassiana*, etc.

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Tailoring diverse microbial consortia for the improvement ofplant nutritional and nutraceutical properties

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The positive impact of microbial consortia on plant growth and health support their use as bioenhancers. The improvement of chicory (*Cichorium intybus* L.) and lettuce (*Lactuca sativa* L.) nutritional value, in terms of nutrient uptake and accumulation of health-promoting compounds, was studied using an in vivo whole-plant system, in different plant genotypes and microbial consortia (MC). The MC were selected on the basis of the occurrence of bacterial strains showing plant growth promoting (PGP) traits in vitro, and of arbuscular mycorrhizal fungi (AMF) capable of enhancing chicory nutrition. The experimental system used allowed the collection of both plant and fungal tissues to determine biomasses and concentrations of nutrients. Moreover, polyphenols, photosynthetic pigments and fructooligosaccharides content and antioxidant activity were assessed in plant tissues. The analysis of nutrients distribution showed that MC often significantly increased iron and zinc contents in plant leaves, and that some micronutrients, namely copper and iron, were accumulated by extraradical mycelium of AMF. The biosynthesis of polyphenols and flavonoids, as well as the antioxidant activity, were enhanced by MC in lettuce and chicory leaves, although differential responses were obtained depending on plant genotypes. The content of fructooligosaccharides, which varied with plant genotypes and MC used, was significantly increased in the chicory var. “Variegata di Castelfranco”, resulting in the accumulation of health-promoting molecules in roots. Overall, data support the use of MC for plant biofortification and for the enhancement of nutritional and nutraceutical value of plant-derived food.

Session 3 – Pathogenic and beneficial aspects of microbes inhuman and animal health

Thursday 19th September, 9:00am

Chairs: Hurtado Ortiz Raquel (France), Susca Antonia (Italy).

Studying host-microbe interactions in health and disease using germ-free and gnotobiotic mouse technology

Jans, Maude[1,5]; Thiran, Alexandra[1,3,5]; Kolata, Magdalena[6,7]; Andries, Vanessa[1,3,5]; Blancke, Gillian[1,3,5]; Ciers, Maarten[1,5]; Thorp, Marie[1,3,5]; Petta, Ioanna[1,3,5]; Diependaele, Louise[1,3,5]; Berx, Geert[2,4,5]; Elewaut, Dirk[1,3]; van Loo, Geert[1,2,4,5]; Remaut, Han[6,7]; Vereecke, Lars[1,3,5]

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The intricate relationship between hosts and their resident microbiota is pivotal to understanding health and disease. Germ-free (GF) and gnotobiotic mouse models are indispensable for elucidating these complex interactions. The Host-Microbiota-Interaction lab (HMI) headed by Prof. Lars Vereecke employs these technologies to uncover mechanisms underlying inflammatory diseases and cancer. We explored the presumed mechanistic link between intestinal inflammation and arthritis, known as the 'gut-joint axis', using transgenic mouse models of gut and joint inflammation [1] Remarkably, germ-free conditions completely prevented intestinal inflammation while joint inflammation persisted, highlighting the microbiota's critical role in gut pathology but not in joint inflammation. These findings suggest that while microbiota targeted therapies could be effective for intestinal inflammatory diseases, joint inflammation may require alternative therapeutic approaches. Further, using unique transgenic models of microbiota dependent colorectal cancer (CRC), we discovered that genotoxic pks+ E. coli strains exacerbate colorectal cancer through adhesion-mediated epithelial binding [2]. Our ongoing research employs these transgenic mouse models to identify and characterize other CRC-associated 'oncobacteria'. Understanding the mechanisms by which these microbes drive disease could lead to targeted therapies that do not disturb the resident microbiota. Collectively, these studies underscore the profound impact of host-microbe interactions on disease progression and health. GF and gnotobiotic mouse technologies are essential for disentangling these interactions, enabling precise studies of microbial influences on various diseases. They pave the way for innovative therapeutic and preventative strategies, leveraging microbiota to improve health outcomes globally.

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Using DNA Metabarcoding as a Non-Invasive Tool for the Conservation of the Critically Endangering Seychelles Bat *Coleura seychellensis*

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Bats constitute a quarter of all mammal species, with many globally threatened or near threatened. *Coleura seychellensis*, an insectivorous bat from the Microchiroptera suborder, is endemic to the Seychelles, restricted to Silhouette and Mahé islands. Once widely distributed, this species is now classified as Critically Endangered. Key factors contributing to its decline include human population growth leading to the loss of lowland forests, clearing of coconut plantations, and habitat modifications due to invasive plants, which have reduced insect populations. Additionally, shifts in the insect community may have disrupted the bat's dietary habits. Accurate and comprehensive knowledge of the feeding habits and ecology of *C. seychellensis* is crucial for understanding its ecological requirements, assessing how food availability affects its population status, and identifying key resources for designing effective management strategies. It has to be considered that diet plays a significant role in shaping gut microbiota, which in turn impacts host health. The present work was aimed at studying the fecal samples of bats in two different seasons with the purpose of (a) describing the bacterial and fungal groups of gut microbiota and (b) investigating the insectivorous diet of *C. seychellensis*. A plastic film was placed under the bat colony in the cave and left for 1 day. Fecal pellets were sampled on the top of the plastic film and placed in DNA/RNA Shield reagent (Zymo). DNA from six pooled samples was extracted using QIAamp PowerFecal Pro DNA Kit (Qiagen). The *C. seychellensis* microbiome and diet were investigated via DNA metabarcoding of the bacterial 16S rRNA gene, fungal ITS2 region and arthropod COI gene. When correlated with knowledge of gut microbial composition, the results obtained from insect-based diet reconstruction could assist in utilizing and addressing microbiome research to conserve endangered species.

Riboflavin overproducing food-grade bacteria: genomic basis, biotechnological applications and perspectives

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Food microbiota is an important reservoir of microorganisms capable of increasing the content ofessential micronutrients, including, among the vitamins of the B-group, riboflavin (vitamin B2), the precursor of the flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD). In Grampositive bacteria the expression of the rib operon for riboflavin biosynthesis is regulated by an FMN-riboswitch consisting of a sensitive domain (aptamer) which, binding to the effector (FMN), induces a conformational change in the regulatory domain. Exposure of vitamin B2-prototrophic strains to roseoflavin (a toxic analogue of riboflavin) allows the selection of spontaneous mutantsable to overproduce riboflavin and harbour mutations in the rib operon riboswitch. In the last twenty years, this strategy allowed the selection of food-grade strains belonging to different species well-known for their technological and/or functional potential. These bacteria are associated withdifferent food matrices and, due to their high metabolic versatility, have been suggested for the sustainable in situ riboflavin bio-fortification of a wide range of fermented foods. Moreover, several riboflavin-overproducing strains have been comprehensively characterised for their probiotic potential, and their administration has been reported to revert ariboflavinosis, as well as to mitigateintestinal inflammatory diseases and some cancers and provide neuroprotective effects. Finally, we discuss preliminary results on future efforts, such as i) to select new strains from spontaneous fermentation and/or unconventional food matrices, ii) to optimise adequate food processing, iii) to elucidate the regulation of the rib operon, iv) to analyse the microbial tolerance against oxidativestress, v) to better understand the beneficial effect of the consumption of B2-fortified foods and/or B2-overproducing probiotics, vi) to exploit strategies to alleviate micronutrient malnutrition in developing countries.

Acknowledgments
We are exploring the potential of this driver of innovation in the framework of the ongoing projects i) the 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] within the Agritech National Research Centre for Agricultural Technologies and ii) the NextGeneration EU [PNRR], in the framework of the Mission 4 Component 2 Investment 1.3-Award Number: Project code PE00000003, Project title: “ON Foods-Research and innovation network on food and nutrition Sustainability, Safety and Security–Working ON Foods”. Vittorio Capozzi was partially funded by CNR project “NUTRAGE FOE-2021 DBA.AD005.225".

Defining the Enterobacter cloacae species complex, with particular emphasis on *Enterobacter hormaechei*.

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Accurate species-level identification within the *Enterobacter cloacae* complex (ECC) is crucial, asthis heterogeneous group is often implicated in nosocomial outbreaks. This precision is essential for microbial bioresource centers (mBRCs), which provide authentic microbial strains and associated information to researchers. Various genomic features, including core-gene phylogenies and average nucleotide identity (ANI), have been used as reliable metrics for defining prokaryotic species.However, the lack of clarity in species definitions often blurs the boundaries, particularly among closely related taxa like ECC members. Recently, *Enterobacter hormaechei* has undergone several taxonomic revisions, revealing a diversity too broad for a single species classification. We sequenced genomes of 12 ECC strains from the Collection of Institut Pasteur (CIP). Phylogenetic analysis based on concatenated sequences of 120 universal (core) genes and pairwise ANI comparisons identified strains corresponding to *E. asburiae*, *E. cloacae*, *E. kobei*, and *E. ludwigii*, alongside distinct clusters of *E. hormaechei*, "*E. hoffmannii*," and "*E. xiangfangensis*." Notably, the latter two are not formally recognized species but are considered subspecies of *E. hormaechei*. Our comprehensive analysis, which included genome sequences of type strains from all *Enterobacter* species and 667 representative strains of *E. hormaechei*, facilitated the circumscription of *E. hormaechei*, "*E. hoffmannii*," and "*E. xiangfangensis*." Additionally, we identified various genesassociated with antimicrobial resistance, including ACT type -lactamase, and virulence factors among the ECC strains. In summary, this study establishes accurate taxonomy for ECC strains and provides strong evidence to delineate species boundaries for *E. hormaechei*, "*E. hoffmannii*," and "*E. xiangfangensis*". It also highlights the utility of population-based approaches, such as genetic discontinuity withinthe populations in substantiating these.

Functionalization of chickpeas through *Saccharomyces cerevisiae var. boulardii*

Accettulli, Alessandra[1]; Corbo, Maria Rosaria[1]; Sinigaglia, Milena[1]; Altieri, Clelia[1]; Bevilacqua, Antonio[1]

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Legumes occupy an important nutritional place due to their wide range of proven beneficial properties; in fact, they provide essential nutrients to our bodies by participating in the eubiotic state of the intestinal microbiota [1]. Probiotic microorganisms are key factors in modulating the microbiota toward eubiosis, and counteract the dysbiosis, that is the disruption of the state of equilibrium that results in the development of various diseases [2].Therefore, the aim of this study is to design a new combination probiotic/matrix. Chickpeas, dueto a greater surface area available, were chosen for the project, while *Saccharomyces cerevisiae var. boulardii* was used as probiotic. In a first step, adhesion experiments were performed to pointout the conditions for a better attachment of yeasts to chickpea surface; while in a second step, the product was thermally treated and then used as a prebiotic-like matrix to assess a potential bifidogenic effect. Finally, a panel test was done. Yeasts were able to adhere at high levels to legume surface, while the addition of thermal treatedchickpeas with yeasts exerted a protective effect on *Bifidobacterium spp*. during the death phase; the organoleptic scores appeared improved.

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Growth inhibition of Human Pathogenic Microorganisms (HPMOs) by endophytic and rhizosphere bacteria

Marangi, Matteo[1]; Szymańska, Sonia[1]; Kęsy, Jacek[1]; Hrynkiewicz, Katarzyna[1]

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In the last decade, the consumption of raw vegetables contaminated with human pathogenic microorganisms (HPMOs) has increased due to poor agricultural processes [1]. The most commonly identified HMPOs were *E. coli*, *Klebsiella spp.* and *Salmonella spp.* and the main vegetable where they were detected was lettuce [1]. The microbial Volatile Organic Compounds (mVOCs) can inhibit the growth of HPMOs. *Bacillus spp.* [2] and *Pseudoalteromonas sp.* [3] were observed to synthesize VOCs against respectively *B. cereus* and *S. aureus*. According to our hypothesis, plant endophytic and rhizosphere soil bacteria can synthesize VOCs that have inhibitory effect onthe growth of HPMOs. The main objective of our work was to select the most efficient bacterial strains to inhibit HPMOs growth. For this purpose, we tested 58 endophytic and rhizosphere bacteria from classes Bacilli, Gammaproteobacteria, Actinomycetes and Alphaproteobacteria (previously isolated from *Salicornia europaea L.*) growing in co-culture with *Listeria monocytogenes, Salmonella enterica* and *Escherichia coli* on bipartite Petri plates. The inhibitory effect of the bacteria was calculated based on the growth of HPMOs compared to the control (bacterial growth in monocultures) after 7 days of culture. A statistically significant inhibitory effect on the growth of HPMOs was shown for 8 rhizosphere soil and 8 endophytic bacteria. Strains that showed high growth inhibition of HPMOs, were selected for solid-phase microextraction mass spectrometry analysis in the headspace (HS-SPME-GC-MS). The HS-SPME-GC-MS revealed the synthesis of some mVOCs with potential anti-microbial activity. Concluding, our study revealed that 16 endophytic and rhizosphere bacteria inhibited significantly the growth of HPMOs and that some ofthem synthesized mVOCs with potential anti-microbial activity.

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Acknowledgments

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Supercritical fluid extraction of torularhodin from a new redyeast strain

Larocca, Vincenzo[1]; Ambrico, Alfredo[1]; Martino, Maria[1]; Trupo, Mario[1]; Magarelli, Rosaria Alessandra[1]; Spagnoletta, Anna[1]

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This study provides valuable informations for recovery of microbial pigments from microorganisms for human and animal health purposes. Some red yeasts, especially by *Rhodotorula* and *Sporobolomyces* genera are potential sources from which to obtain carotenoids. In this work a new strain of *Rhodotorula* (ELP2022), currently present in the ENEA Microbial Culture Collection (EMCC), was isolated from fresh cheese and characterized for its metabolic pattern (Biolog, Inc)and carotenoids profile. The main carotenoids identified were: -carotene, -carotene, torulene and torularhodin. The latter is a dark pink coloured carotenoid belonging to the xanthophylls group and the growing interest in this molecule is due to its biological activities such as antioxidant, anticholesterolemic, anti-inflammatory, antimicrobial and anticancer. In this context the strain ELP2022 was used to develop a new efficient method for selective extraction of torularhodin in two sequential steps by applying the extraction technique with supercritical CO2 (CO2-SFE) andthe method has been the subject of an Italian patent deposit (n. 102023000018729). In particular, it was grown in bench-scale fermenters using glucose-based mineral media. The pre-treated and dried biomass was subjected to a CO2 extraction process in supercritical conditions (CO2-SC) bya bench scale system (Applied Separations Spe-ed SFE-2). After extraction the torularhodin represented no less than 95.2% of the total carotenoids in the red extracts obtained from the second step. Therefore, this method allows to separate torularhodin from other carotenoids without the use of chromatographic techniques and doesn't involve the use of solvents potentially dangerous for health and environment. So, this approach avoids the presence of residual solvents of class 1 and 2 in the extracts, which should be respectively not employed or limited in pharmaceuticals according to ICH guidelines Q3C(R8).

Acknowledgments
This work was granted by the European Commission NextGenerationEU within the framework of the National Recovery and Resilience Plan (Mission 4 "Education and Research", Component 2, Investment 3.1), Project "Strengthening the MIRRI Italian Research Infrastructure for Sustainable Bioscience and Bioeconomy" (SUS-MIRRI.IT), code IR0000005.

Interaction between probiotic lactic acid bacteria and immunosuppressive drugs

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The human microbiota consists of trillions of microorganisms living in a commensal state in different niches of the body, of which the gut is the most studied. In some specific diseases, importantchanges in the composition of the microbiota occur as a consequence of the disease itself or the therapy taken by the patient. A prototypical example of such a clinical condition is the kidney transplant. After kidney transplantation, important changes in the composition of the gut microbiota have been observed, with an increase in Firmicutes, Proteobacteria and Bacteroidetes. These changes can lead to infectious complications and influence the synthesis of uremic toxins, as well as alter the response to immunosuppressive drugs commonly used during kidney transplantation therapy. Studies in the literature show that the use of probiotics can modify and/or improve the state of intestinal dysbiosis. Therefore, the aim of this study was to evaluate the interactions between different probiotic lactic acid bacteria and immunosuppressive drugs commonly administered in post-transplant therapy. In detail, different strains of probiotic lactic acid bacteria belonging to the species *Lactiplantibacillus plantarum*, *Lacticaseibacillus rhamnosus*, *Lactobacillus acidophilus*, *Lacticaseibacillus casei*, *Lacticaseibacillus paracasei*, *Limosilactobacillus reuteri*, *Streptococcus thermophilus* and immunosuppressive drugs Mycophenolate Mofetil (MMF), Mycophenolic Acid (MPA) and Mycophenolic Acid Glucoronide (MPAG) were investigated. The effect of the bacterial-drug interaction was assessed by analysing the viability of the microbial species during an incubation time of 24h at 37°C under anaerobic conditions. Furthermore, the ability of probiotic lactic acid bacteria to metabolise and/or accumulate immunosuppressive drugs in vitro was assessed by HPLC analysis.

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Factors affecting the production of y-aminobutyric acid in *Levilactobacillus brevis* LB12

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Y-aminobutyric acid (GABA) provides several physiological benefits to human health [1,2]. Lactic acid bacteria (LAB) are recognized as potential GABA producers and several strains, mainly belonging the species *Levilactobacillus brevis*, have been investigated and characterized for their ability to produce GABA in synthetic media and/or food matrices [1,2]
In this study, the effect of different pH (3.5, 4.0, 4.5, 5.0, 5.5, 6.0; buffer system), glutamate concentration (0, 1, 2, 5, 10 g/L of monosodium glutamate, MSG; buffer system) and atmosphere of incubation (cultivation and adaptation to anaerobic and aerobic conditions; AN vs AE) was evaluated on the GABA production in *Lvb. brevis* LB12. Glutamate (Glu) consumption and GABA accumulation was estimated with Thin Layer Chromatography and densitometric spot analysis [3]. The expression of GAD operon genes (gadB/gadA; glutamate decarboxylase, gadC, glutamate: gamma-aminobutyrate antiporter; gadR, transcriptional regulator, gltX, glutaminyl-tRNA synthetase), in response to the atmosphere of incubation and Glu supplementation, was evaluated byusing RT-qPCR (SYBR Green protocol). The optimal pH for Glu uptake and GABA production was 4.5, while the greatest inductive effectof Glu supplementation was observed with 10 g/L MSG. AE conditions increased biomass yield, but impaired the efficiency of Glu to GABA biotransformation, resulting in a lower GABA accumulation, in both culture supernatant and reaction buffer. The expression of genes belonging to GAD operon (gadB, gadC, gadR) was also impaired in AEgrowing cells. gadA gene, 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 efficiencyof Glu-GABA conversion in *Lvb. brevis* LB12.

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Comparison of microbial populations of peritonsillar abscess determined by molecular and bacteriological methods

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Peritonsillar abscess (PTA) is a severe deep neck space infection with an insufficiently characterized bacterial etiology. We aimed to reveal bacteria associated with PTA applying next generationsequencing (NGS) and traditional bacteriology.
The study group consisted 113 consecutive patients with PTA. Pus and tonsil biopsies were investigated. Semi-quantitative bacteriological methodology was used for the examination of all samples. Colonies were identified by MALDI-TOF mass spectrometry. NGS method was additionally applied to samples of 91 PTA patients. Over 400 genera and 800 species belonging to 34 phyla were revealed by NGS. The most abundant species in both sample types were *Streptococcus* pyogenes, *Fusobacterium necrophorum* and *Fusobacterium nucleatum*. Other species displaying a high mean relative abundance were *Prevotella oris* in all samples; *Porphyromonas endodontalis* in pus and Streptococcus mitis in tonsils. *S. pyogenes* and *F. necrophorum* were the predominant species (> 10% in a community) in 31% pussamples, while *F. nucleatum* in 23% and *S. anginosus* in 9% pus samples. In total, 174 bacterial isolates were identified, which belonged to 5 phyla, 25 genera and 62 species. In the tonsil biopsies, the most frequent isolates were *Streptococcus parasanguinis* and *Streptococcus pneumoniae* (both 10.5%), followed by *Neisseria spp* (16.6%) and *Actinomyces spp* (7%). In pus samples, the most frequently isolates were *Streptococcus spp* (48.3%). *S. pyogenes* and *F. necrophorum* was isolatedfrom both type samples while *F. nucleatum* only from pus. 156 bacterial strains were deposited tothe Human Microbiota Biobank (HUMB).
The most probable causative agents of PTA are *S. pyogenes*, *F. necrophorum* and *F. nucleatum*. Some streptococci (*S. anginosus*, *S. parasanguinis*) and anaerobes (*Prevotella*, *Porphyromonas*) may contribute to the infection as well. Pus of the abscess is more representative specimen for examination. All deposited strains are available for future research purposes.

Acknowledgments
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Use of potential probiotic autochthonous *L. plantarum* as co-starter for the production of goat's milk cheeses

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Raw milk and artisanal cheeses are characterized by a microbiota mostly constituted of starter and non-starter lactic acid bacteria (NSLAB), the latter playing a key role in the ripening and flavour development of the cheeses. Mesophilic lactobacilli are the predominant group in the microbiota of NSLAB and are endowed with probiotic potential. Cheese has been proven to be an optimal carrier product to deliver living probiotic bacteria, and autochthonous strains with potential probiotic features would be the best choice for use as adjunct cultures since they should be well-adapted to this food environment. In this study three autochthonous *Lactiplantibacillus plantarum* strains possessing several probiotic and technological properties were used as co-starter cultures in the manufacturing of goat's milk cheeses. The aim of the study was to evaluate the presence and viability of these strains during maturation and ripening of goat's milk cheeses producedat industrial scale-level, using both conventional culturing and PCR-DGGE culture-independent technique. Compositional, microbiological, and sensory analyses were also carried out to evaluatethe impact of the *L. plantarum* strains on the cheese's quality. The results showed that lactobacillicount evolved similarly in the cheeses produced with the three autochthonous strains, maintaining high viability until the end of ripening (>9 log10 cfu/g in 30-days-cheeses). DGGE profiles showed a band corresponding to *L. plantarum* in the cheese samples throughout the whole ripening period. No significant differences in the physico-chemical parameters were found between the cheeses produced with the three different autochthonous cultures. Sensory evaluation indicated that the use of the autochthonous potential probiotic co-starter did not adversely affect the overall acceptability of the goat cheeses, however, those produced with the strain *L. plantarum* 11/20966received significantly higher scores for odour, aroma, and texture.

Acknowledgments
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Session 4 – Microbial life in extreme habitats: a 21st century challenge

Thursday 19th September, 11:00am

Chairs: Varese Giovanna Cristina (Italy), Sedláček Ivo (Czech Republic).

Diversity of microalgae in low pH environments: fifty years of the ACUF Collection, from strain to microbiome conservation and exploitation.

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ACUF (Algae Collection of the University of Naples Federico II) was founded in 1974 as a group ofstrains collected by Professor Taddei and his collaborators to study the ecophysiology of the thermoacidophilic unicellular red alga *Cyanidium caldarium*. The strains were initially collected from the Italian peninsula and Sicily, in low pH environments (hot springs, fumaroles, sulphur mines) and have been used in numerous physiological, ecological and morphological research projects. Since 1978, the ACUF has held more than 300 C. caldarium strains and was established as a facility of the Istituto di Botanica of the University of Naples. In the following decade, new accessionswere deposited by other researchers from the Istituto di Botanica, who explored low pH environments in America and Asia in search of Cyanidiales populations. Since then, ACUF members have visited other countries on different continents, extending the sampling to different types of extreme environments, such as dry rocks and caves. ACUF is a member of the World Federation for Culture Collections and an associate member of the Italian Microbial Resource Research Infrastructure (MIRRI-IT). The functions of ACUF are to collect and maintain in culture isolates ofalgae and cyanobacteria, mainly from extreme environments, to serve as a repository and to make these isolates and related information available for research and biotechnology applications. The ACUF collection contains 112 genera and about 1000 isolates, including cyanobacteria. Stocks ofmost cultures are maintained as live strains, but part of the isolates are also maintained in nongrowing conditions, frozen with or without cryoprotectants. Under the framework of the PNRR project SUS-MIRRI, management practices to maintain and cultivate microbiomes from low pH environments are in progress, as well as co-culture mass cultivations, in which bacteria and microalgae collected from the same site are grown together to improve productivity and biosynthesisof valuable compounds.

Life at the limits: diversity, adaptation strategies and bioprospecting of microbes living in Arctic deep-sea habitats (INDEPTH)

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The deep-sea biosphere represents the largest living system on Earth, distinctive in its unparalleled complexity. This vast biosphere, often referred to as life's last frontier, is characterized by darkness, high pressure, limited nutrients, and a wide range of temperatures from low (2-4°C) in most habitats to extremely high (above 100°C) in the seabed hot hydrothermal vents. The INDEPTH project aimed to decipher the hidden reservoir of metabolic traits of the unique biodiversity at the Arctic Mid-Ocean Ridge (AMOR) vents. By reconstructing and analyzing AMOR microbial genomes, the potential for homoacetogenesis via the Wood-Ljungdahl pathway (WLP) was elucidated in the archaeal Korarchaeia, supporting previous hypotheses that the WLP has evolved independently from the methanogenic metabolism in Archaea [1]. In parallel, a large metagenomic dataset, comprising individual assemblies from 14 samples from Loki's Castle, Perle and Bruse, and Soria Moria vent fields, formed the foundation for developing a novel biodiscovery pipeline to identify novel enzymes with unique activities [2]. The project targeted DNA processing enzymes, proteases, cellulases, betagalactosidases, laccases, alkaline phosphatases, and chitinases. As a result, more than 30 novel enzymes have been thoroughly characterized at both structural and functional levels [3]. The INDEPTH project expanded our understanding of biodiversity, evolution and the functioning of complex microbial communities, including adaptation strategies required for survival in theextreme conditions of seafloor hot vents. It also led to the development of new tools for metagenomics that enhance bioprospecting and deepen our understanding of microbial life in extreme Arctic habitats.

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Cold-adapted carboxylic ester hydrolases from two Antarctic *Psychrobacter* strains: genomic analyses and in-vitro studies

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Pollution from polyester plastics is increasingly affecting biotic and abiotic components of the ecosystems, making it necessary to find effective solutions. The application of bacterial enzymes–mediated degradation is a promising strategy to treat plastic waste. Therefore, extremophiles and extremozymes could be applied in bioremediation due to their peculiar features of adaptation to these ecological niches.
Psychrobacter strain ASPA161_6 and strain ASPA161_9 were isolated from marine sediment samples collected in Terra Nova Bay (74°44'854"S, 164°05'223"E; Antarctica), from the Extremophiles Research Group of Biomolecular Chemistry Institute of the National Research Council (CNR), Pozzuoli (IT), and are stored in the Extremophiles Collection (CE-ICB) (www.susmirri-catalog.di.unito.it/collections) part of the SUS-MIRRI project, which collects, characterizes, and distributes microbial strains for research and bio-industrial purposes preserving microbial biodiversity. Their genomes were sequenced and deposited at DDBJ/ENA/GenBank with accession numbers JBEFNJ000000000 and JBEFNI000000000, respectively. The digital DNA–DNA hybridization and Average Nucleotide Identity were calculated.
After the functional annotation of the genomes with the RAST Server, *in silico* screenings were performed, highlighting coding sequences related to low-temperature adaption mechanisms and to shock response. Carboxylic ester hydrolases activities and enzymes potentially involved in polyester material degradation were researched. Wet-lab experiments were set up to recover esterase (EC 3.1.1.1) and lipase (EC 3.1.1.3) enzymes from the supernatants of the two strains. The detection of non-specific carboxylic ester hydrolases was done through qualitative agar plate-based assays, while quantitative in-vitro spectrophotometric assays defined the specificity of these enzymes onto classes of substrates, with the outlook to catalyze the depolymerization of plastic under competitive conditions.

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Cold-adapted yeasts: a restricted club of extremophilic organisms

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Cold habitats (average temperature < 5°C) represent over 80% of the Earth's environments. Theseinclude the Arctic and Antarctica, high mountains in Asia, Europe, and America, cold deserts, and the deep sea. Although their harsh environmental conditions, cold habitats support both psychrophilic and psychrotolerant fungi (including yeasts), which have developed specific adaptations to cope with low temperatures, e.g. the decrease in growth rates, the synthesis of cold-active enzymes and of cryoprotectant molecules (e.g. cold-shock proteins, sugars, polyols), and an increased fluidity of cell membranes at low temperatures due to the presence of unsaturated fatty acids in their lipid fraction.
The impact of global warming on terrestrial cold habitats is amplifying every year by increasing the duration of ice-free periods. Therefore, studying the diversity of fungal communities (both yeasts and filamentous fungi) occurring in polar and Alpine cold areas may be considered strategic for enhancing our knowledge of the fungal ecology of these understudied niches.
DNA metabarcoding studies (i.e. NGS targeting the ITS fungal region) on fungal communitiesof soil (including permafrost), brines, ice and debris were conducted to evaluate the taxonomic structure of fungal communities and their ecological interaction with abiotic parameters. Overall,taxa (classified at the genus level) ascribed to Ascomycota dominated among filamentous fungi, while Basidiomycota dominated among yeasts. At the phylotype level, yeasts were predominant in the fungal communities. Alpha and beta-diversity analyses revealed high fungal richness andtaxonomic differentiation among samples, even at the small-scale level. Salinity, conductivity, pH and the concentration of organic carbon had a significant ($p < 0.05$) impact on the abundance of some fungal and yeast taxa. Yeasts culturable diversity reflected the results of metabarcoding analysis: an overall dominance of Basidiomycota was confirmed.

Preliminary investigations of alkali-tolerant fungi in stromatolites from Lake Salda (Burdur province, SW Türkiye)

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Despite that fungi prefer to colonise neutral or slightly acidic environment; it has been demonstrated that some of them can survive and thrive at high pH. This is due to fungal productionof alkaline enzymes like proteases and amylases which protect fungi [1]. These enzymes can be exploited in various industries and used for biotechnological purposes. Therefore, alkaliphilic and alkali-tolerant fungi seem to be of particular interest here.
Lake Salda is a highly alkaline (pH>9) and magnesium-rich lake, located in the Burdur province (SW Türkiye). The lake is one of the deepest enclosed lakes in Türkiye and surrounded by ultramafic and karstified carbonate rocks. Large stromatolitic bodies, fossil and living, built of hydromagnesite, controlled by biological processes, are abundant here. In November 2023, samples from living stromatolites were collected in the littoral zone of Lake Salda. Samples were analysed to obtain their physico-chemical and mycological characteristics. Chemical analysis of lake watershowed presence of 0.21 mg/L PO_4, 0.02 mg/L Fe, 2.4 mg/L NO_3-N, 0.009 mg/L NO_2-N, 84.9 mg/L Cl, 0.33 mg/L NH3-N, 0.65 mg/L Mg, and 0.18 mg/L Ca, whereas mycological analysis revealed a total of 20 strains, which were isolated from the stromatolite samples and conserved inthe culture collection (ColD UNIGE JRU MIRRI IT). Many alkali-tolerant fungal genera and species have been identified such as *Acrostalagmus luteoalbus*, *Alternaria* sp., *Aspergillus templicola*, *Curvularia* sp., *Emericellopsis* sp., *Fusarium equiseti*, and *Stachybotrys cartarum*. Further investigations will be carried out not only to study the potential production of alkaline enzymes and interesting metabolites from these fungi, but also to deepen our understanding of the fungal role in hydromagnesite precipitation and stromatolite-building mats.

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From microbial diversity of Campania thermal springs of their maintenance and preservation

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The lithosphere is enriched by significant amounts of volatile elements: carbon, oxygen, hydrogen. These elements are often released through subduction zones, where geosphere and biosphere interact with another. The recycling of these volatile substances plays a fundamental role in fueling subsurface microbial communities [1]. Much remains to be understood about the relationship between volatile delivery and microbial composition. The main Italian geothermal activity originates from the subduction of the African and Adriatic plates beneath the Eurasian plate makes the peninsula an excellent area to investigate these relationships. This process generated severalsecondary geothermal emissions, generating unique, in some cases extreme, geochemical compositions. For this purpose, we analyzed fluid samples collected from 13 geothermal springs present in the Campania region [2]. By combining geochemical and biological data we are able to provide an overview of the physicochemical extremes imposed by the tectonic setting on the subsurface biosphere. Knowing the role of major, minor, and trace elements in influencing the deep biosphere is necessary to the further understanding of the processes linked between the coevolution of life and geology. In turn, this will be helpful to search for the presence of such processes in other small rocky planets with Earth-like tectonic activity. In addition, due to the current technical andphysiological limitations of non-cultivating 90% of microorganisms, enhancing preservation and culture methods to maintain and investigate complex communities alive become crucial.
To this end, we are currently engaged in the development of SOPs [3] aimed at microbiome cultivation through the use of the Winogradsky column technique and preservation strategies using different cryoprotective agents coupled with different freezing strategies. This work will lay the bases to constitute the first Italian collection dedicated to microbiomes from extreme environments

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The influence of prokaryote communities on *Galdieria sulphuraria* strain maintenance and cultivation

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The Algal Collection at the University of Naples Federico II (ACUF) is a biological resource center that maintains microalgal strains, primarily from Cyanobacteria, Chlorophyta, Rhodophyta, and Bacillariophyceae. A resource of ACUF collection are extremophilic algae mostly belonging to the Cyanidiophytinae isolated from European and non-European sites. The Cyanidiophytinae are a subdivision of the Rhodophyta including species that can survive in thermal and hyperacidic conditions up to 56 °C and a pH lower than 2.0 [1]. They represent the only phototrophic and eukaryotic organisms found in geothermal sites under the above mentioned conditions, in close association with extremophilic bacteria and archaea [2]. Cyanidiophytina members, such as *Galdieria sulphuraria*, are considered the most promising microalgal species for future biotechnological applications (food, health and cosmetics). Interactions between Cyanidiophytinae and prokaryotes cover the full range of symbiotic relationships considered possible. However, most of the interactions between these microalgae and their prokaryotic partner are still poorly studied because separation is a challenging task [3]. This study presents preliminary results from sequencing and culture approaches aimed at exploring the microbial diversity associated with *Galdieria sulphuraria* samples collected at the Mefite d'Ansanto (southern Apennines, Italy). The variation in microbial community composition over time was studied using samples collected in different years. To investigate the interactions between the primary microbial groups and potential metabolic exchanges, selected samples were cultivated under photoautotrophic conditions. Following DNA extraction and sequencing, bioinformatic analyses were conducted to further examine these interactions.

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Cryopreservation strategies for extremophilic microalgae at ACUF: growth kinetic and photosynthetic assessment

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Ex situ conservation allows to preserve genetic materials and functional characteristics of microalgae against environmental and ecological change that could cause the extinction of previously isolated organisms over time [1]. Microalgae conservation is also the first step for any kind of biotechnological exploitation [2]. The present work aims to illustrate the strategies of cryopreservation for extremophile and extreme tolerant strains belonging to The Algal Collection at the University of Naples Federico II (ACUF) within the framework of PNRR_IR SUS-MIRRI.IT project. *Chlorophyta* and *Rhodophyta* are among the largest groups of photosynthetic microbes maintained at ACUF [3] deserving tailored strategies of preservation [4]. We first focused on conventional protocols suitable for cryopreservation of both extreme tolerant and extremophilic microalgal strains,and also tried to find novel methods. Two strains were selected for testing: one belonging to *Stichococcus bacillaris* Nägeli (*Trebouxiophyceae*, *Chlorophyta*), an acid tolerant unicellular green alga commonly cryopreserved, and the other to *Galdieria sulphuraria* (Galdieri) Merola (*Cyanidiophyceae*, *Rhodophyta*), a thermo-acidophilic microalga isolated from an italian sulphur spring. The extremophilic strains of *Galdieria* have proved difficult to conserve using cryogenic methods.Different cryopreservation strategies applied on two strains belonging to *Stichococcus* and *Galdieria* were compared to evaluate the adaptability of these strains to cryopreservation. A novel variable is introduced to assess viability recovery [5]. Photosynthetic rate and pigments assessment were measured to evaluate hidden metabolic cell damage.

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Fermentation Technologies for the Optimization of levan Production by *Pseudomonas* strain 2ASCA

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Pseudomonas 2ASCA is an Arctic Gram-negative psychrophilic bacterium isolated from Extremophiles Research Group of Institute of Biomolecular Chemistry of the National Research Council (CNR) in Pozzuoli (Italy) and it is stored in Extremophiles Collection (CE-ICB) (www.susmirri-catalog.di.unito.it/collections) included in the SUS-MIRRI project [1].
Strain 2ASCA produces a homopolymeric exopolysaccharide loosely bound to the cell membraneconsisting of a long-chain -(2,6)-linked fructose polymer, called levan. A crucial aspect of largescale microbial production of exopolysaccharides (ESPs) is the costs associated with production and recovery. For this reason, to optimize the growth of the strain and the hyper-production of levan, different substrates were tested as main carbon sources. Its ability to utilize many sugars as the sole carbon source has driven the search of exopolysaccharide in two different cellular fractions, in loosely and tightly bound fractions (LB and TB, respectively) and in cell-free supernatant fractions (S). Exopolymers obtained from S, TB, and LB fractions were tested for the content of extracellular polysaccharide substance (EPS), proteins, and uronic acids. Among the substrates tested, sucrose supported the best cell growth and was the most suitable carbon source for the induction of the polysaccharide pathway, resulting in the release of the exoproduct consisting of a high percentage of carbohydrates and a low percentage of proteins and uronic acids. Moreover, levan polysaccharide was produced by replacing pure sucrose with agro-industrial waste to achieve sustainability and circular economy objectives [2]. In fact, bacterial fermentation with molasses from sugar beet processing as the sole source of sucrose led to significantly amplified productionof EPS. The increase in EPS yield was, most likely, due to the presence of nitrogen and mineral components in the molasses, compared to the sucrose-containing minimal growth medium.

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Extremophiles Collection of Institute of Biomolecular Chemistry National Research Council (CE-ICB CNR): microbial biodiversity preservation of extreme environments within the SUS-MIRRI.IT project

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Extremophiles, including thermophilic, halophilic, haloalkalophilic, psychrophilic, and polyextremophilic bacteria, are found in diverse habitats such as hydrothermal vents, salt lakes, polar regions, volcanic areas, and deserts [1]. Their adaptive strategies, involving various cellular metabolic mechanisms, led to the production of unique biomolecules with industrial applications in several biotechnology sectors [1,2]. Their ability to thrive under extreme conditions makes them valuable for astrobiology and assessing planetary habitability [2]. Extremophiles Research Groupof Institute of Biomolecular Chemistry of the National Research Council (CNR) in Pozzuoli (Italy),through international collaborations and research projects, has sampled extreme niches, isolated and characterized microorganisms that are available to the scientific community and stakeholders as Extremophiles Collection (CE-ICB) (www.susmirri-catalog.di.unito.it/collections), included in the SUS-MIRRI project, which collects, characterizes, and distributes microbial strains for research and bio-industrial purposes preserving microbial biodiversity [3]. In fact, natural resource exploitation, climate change, and human activities are accelerating ecosystem destruction and microbial biodiversity loss, which means losing potential biological tools for global challenges. In addition, extremophilic biomass and its molecules (enzymes, exopolymers, lipids, etc.) can be hyper-produced by the bioprocess facility present at ICB-CNR. The availability of several sized bioreactors (www.icb.cnr.it/institute-facilities/bioprocessi) allowed the study of biotechnological potential of such biomolecules in terms of valorization of agro-industrial wastes, biodegradation, bioremediation, cultural heritage preservation, and agriculture [4,5,6]. Recently, *in silico* genome studies furnished an innovative tool for investigate the functional prediction and subsequently thepotential biotechnological uses of Extremophiles [7].

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Exopolysaccharides from psychrophilic microorganisms as biological device for plant promotion.

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Psychrophiles can grow and multiply at temperatures between 0°C and 20°C [1]. They are presentin Alpine, Antarctic and Arctic environments, and have been isolated at high latitudes, in deep ocean waters, polar glaciers and snowfields. They are characterized by the presence of lipids in their cell membranes that are resistant to the rigidity caused by cold and often form antifreeze proteins to keep liquid their extracellular or intracellular space protecting the integrity of their cells even at temperatures below the freezing point of water. In addition, they secrete exopolysaccharides (EPS) that, surrounding the producer cells, represent a protective barrier against extreme low-temperature conditions. Some microbial EPS promote root colonization and retaining waterform a hydrophilic biofilm. These polysaccharides form capsules or coatings around cellular surface and can aid in cell adhesion, environmental adaptation, uptake of nutrients and stress tolerance.Some psychrophiles that produce EPS are *Colwellia psychrerythraea*, *Pseudoalteromonas* sp., and *Olleya marilimosa*. An exopolysaccharide currently studied for plant growth promoting properties (PGP) determination is levan (Lev-2ASCA), produced the psychrophilic bacterium *Pseudomonas* 2ASCA [2]. In addition, it has been proved that Lev-2ASCA efficiently chelates heavy metals, with interesting detoxifying effects of the soils in which it could be applied [3]. Therefore, experiments in pots with barley plant treated with Lev-2ASCA are ongoing, in frame of project PRIN 2022LPPFTY, -TREASURE. This strain, isolated from Extremophiles Research Group of Institute of Biomolecular Chemistry of the National Research Council (CNR) in Pozzuoli (Italy), is stored in Extremophiles Collection (CE-ICB) (www.susmirri-catalog.di.unito.it/collections) included in the SUS-MIRRI.IT project, which collect, characterize, and distribute microbial strains for research and bio-industrial purposes preserving microbial biodiversity.

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Genome Prospecting and Wet-Lab Evidence of Plant Growth Promotion Traits in the Psychrophilic Arctic Bacterium *Pseudomonas* strain 2ASCA

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The shift towards eco-sustainable techniques in agriculture is crucial to minimize the use of phytochemicals such as fertilizers and pesticides. In this context, innovative solutions like microbe-based biofertilizers are at the forefront. The PRIN project "TREASURE" explores *Pseudomonas* strain 2ASCA, an Arctic bacterium, for sustainable agriculture, focusing on plant growth promotion (PGP). P. 2ASCA is known for producing the polysaccharide levan, significant for its biotechnological applications [1]. The levan-based biofilm matrix could also be crucial for the successful inoculation of P. 2ASCA as a biofertilizer, enhancing its efficacy in promoting plant growth. *In silico* genomic analysis revealed genes (e.g., glnA, aconitate hydratase 2, catalase KatE) crucial forPGP, aiding nitrogen assimilation, energy production, and stress tolerance [2]. Additionally, genes for nutrient solubilization, siderophore biosynthesis, and ACC deaminase were identified, enhancing plant nutrient uptake and stress resilience. *Pseudomonas* 2ASCA also encodes enzymes for plastic degradation (e.g., LDPE, PHBV, PHA, PLA, and PCL), suggesting roles in agricultural sustainability and plastic pollution mitigation [3]. Experimental validation involved qualitative screening of direct and indirect PGP traits [4]. The bacterium displayed significant ACC deaminase activity, particularly at 15°C, with reduced activity at 5°C. IAA production was moderateat both temperatures, and organic acid production was slightly positive. Notably, P. 2ASCA was positive for siderophore production, indicating its biocontrol potential. Further studies are ongoing to experimentally investigate additional PGP traits and to deeply characterize the PGP features already identified, aiming to harness P. 2ASCA's capabilities for biotechnological applications insustainable agriculture. This strain belongs to Extremophiles Collection (CE-ICB) (www.susmirricatalog.di.unito.it/collections) included in the SUS-MIRRI.IT project.

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Acknowledgment
This work was partially financed by project PRIN 2022LPPFTY, -TREASURE, and by FOE-CNR FutuRaw Project.

Biosynthesis of Nanoparticles by Extremophilic Bacteria for Potential Biotechnological Application

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Nanoscience and nanotechnology attract a great interest due to their potential impact on many areas such as energy, medicine, pharmaceutical industries, electronics, etc. This technology deals with small structures and small-sized materials of dimensions in the range of 1100 nm. Nanoparticles (NPs) show unique physical and biological properties, due to their high surface-to-volume ratio. Methods chemical and physical for the synthesis of nanoparticles, are considered unfavourable due to high capital cost, use of toxic reagents and the generation of hazardous wastes. Synthesis of nanoparticles by biological means offers cheap, non-toxic and eco-friendly alternative to the common physical and chemical methods. The use of biological samples like bacteria, plant extracts, fungi, polysaccharides and enzymes for the synthesis of NPs, provides various advantageslike eco-friendliness and compatibility for biomedical and other pharmaceutical applications. In this frame, we used extremophilic bacteria to produce biogenic AgNPs and SeNPs. AgNPs were produced extracellularly by means of the thermophilic *Thermus thermophilus* strain SAMU; the haloalkaliphilic *Halomonas campaniensis* strain 5AG was used for the intracellular synthesis of SeNPs. The structural characterization was carried out by means of UV-visible spectroscopy, dynamic light scattering (DLS), transmission electron microscopy (TEM), Fourier transform infrared spectroscopy (FT-IR) and zeta potential. Both the Ag and SeNPs possessed a protein coatingon their surface, they were organized in aggregates and showed antibacterial properties versus *Escherichia coli* DSM 648 and *Kokuria rhizophila* DSM 348. In addition, levan-type polysaccharide from psychrophilic *Pseudomonas* strain 2ASCA, develops particles in acidified water with heavy metal chelating capability revealing a strong affinity for Cr(III), never reported before for microbial levans. This behavior was studied using DLS and scanning electron microscopy (SEM).

Acknowledgment
This work was funded by CNR project FOE-2021 NutrAge—code DBA.AD005.225
This research was partially supported by the PNRR European Commission – NextGenerationEU, Project "Strengthening the MIRRI Italian Research Infrastructure for Sustainable Bioscience and Bioeconomy, SUS-MIRRI.IT", code n. IR0000005". This work was partially supported by PRIN 2022LPPFTY

The Haloarchaea Morphological Atlas discloses animal-like tissue structures emergence in single-celled archaea

Theopi Rados[1]; Olivia Leland[1]; Pedro Escudeiro[2]; John Mallon[1]; Ido Caspy[3]; Elad Stolovicki[4]; Inés Patop[1]; Sebastian Kadener[1]; Vera Thiel[5]; Yoav Soen[4]; Tanmay Bharat[3]; Vikram Alva[2]; Alex Bisson[1]

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With our large collection of >100 haloarchaea, the Leibniz Institute DSMZ joins a comprehensive evolutionary developmental biology project with international researchers studying the biophysical and spatio-temporal control of unusual cell shape transitions across salt-loving archaea using state-of-the-art microscopy from single-cell and single-molecule tracking, microfabrication, and genetics, building an unprecedented “Haloarchaea Morphological Atlas”. It is hypothesized that together with reading out their chemical environment, archaeal cells evolved to sense physical cues to build different cell shapes and mediate social behavior within the same and across different species, which is studied as part of the project. To explore this idea, the mechanisms of mechanosensing in Archaea, the closest single-celled prokaryote (cells without a nucleus) to animals, are investigated.Surprisingly, archaeal cells react to confinement and compression with the development of a tissue-like state, with cells physically connected, resembling the structure of an epithelial monolayer. It was shown that this multicellular development depends not on material stiffness but on axial compression. In the first hours after being compressed, the cells grow larger without cell division but with accelerated DNA and protein synthesis. After reaching a precise cell volume, cell division is once again reinstated, but now, instead of separating, cells remain attached by cell-cell junctions.In the ongoing project, it will be further addressed which specific factors respond to mechanical compression and which factors contribute to developing and maintaining the multicellular structures. This novel and unexpected discovery introduces a new way of understanding and studyingtissues in a simpler and straightforward model organism.

Session 5 – Microbiomes preservation and exploitation

Wednesday 18th September, 3:00pm

Chairs: Ferrara Massimo (Italy), Aznar Rosa (Spain).

Microbiomes for the Industry

Porcar, Manuel[1]

[1] *DARWIN BIOPROSPECTING EXCELLENCE, Valencia, Spain*

Our planet is home of a large biodiversity of microorganisms, which do not live alone but with a dynamic interaction with their tiny neighbours. Bioprospecting, that is, the search, characterisation and selection of microbial taxa, can lead to the use of microbiomes and simplified consortia for the food industry. In particular, simplified consortia can be very useful for improved sourdough baking; for yielding novel fermented dairy products with organoleptic and/or functional new properties; and can also be the preferred strategy for brewing alcoholic and non alcoholic beverages that combine industrialisation suitability and organoleptic value. Bioprospecting microbiomes can also lead to develop non food, environmental biosolutions, such as fostering biogas production through bioaugmentation and digestate transplantation; or plastic biodegradation and sulphate reduction in polluted wastewater. Besides culturomics, microbiomes are also a source of genes and enzymes with potential in biomedicine and bioremediation. Culture-independent, genomic mining is thus also a valuable tool to develop microbial solutions with biotechnological potential. Finally, bioprospecting lies on the interphase between industrial applications and social benefits, and theimplications of such interaction in the frame of the Nagoya protocol will be discussed.

Exploring soil microbiome preservation strategies: culturablefraction, metabolic profiling and metagenomics

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Holistic approaches are required to better understand the distribution of microbial diversity andthe functional profile of communities in soil microbiomes. In the present work we investigated how different sample preservation techniques can affect the soil rhizosphere microbial communities by analysing the culturable fraction of microorganisms, the microbiota and the metabolic fingerprint. Three different storage conditions were considered: refrigeration at 4°C, freezing at -80°C, and lyophilization. Six rhizosphere samples from strawberry, grapevine and kiwifruit plants grown intwo field soil types were analysed. Microbial community structures and growth strategies were investigated by means of total microbial count, enumeration of copiotrophs and oligotrophs bacteria, r-k strategists and eco-physiological (EP) index. A total of 300 bacterial strains and 315 fungi were isolated for further investigation of plant growth promoting traits. Functional-level metabolic profiling of microbial communities based on Biolog EcoPlates highlighted widespread carbon sources utilisation in samples with high population diversity, confirmed by metagenomic analysis, such as kiwifruit and strawberry, when compared to poor species abundance soils, as grapevine. Linking the distribution of microbial diversity and ecosystem functioning is essential to understand community responses to changing environment, especially in forced conditions such as samples preservation. For that reason, the same analysis will be performed on samples at 6 and 12 months and results will help better understand structural shifts during microbiome conservation and which functional features in the community can be affected during long term storage. In parallel, within isolates showing PGP traits, microbial consortia set-up will be carried out to exploit natural inhabitants of soil communities (NatComs) as biofertilizer to enhance crop productivity.

Acknowledgements
The authors thank Luca Cocolin for his valuable support. This activity has received funding from the SUS-MIRRI.IT Project “Strengthening the MIRRI Italian Research Infrastructure for Sustainable Bioscience and Bioeconomy”, Project code IR0000005, CUP D13C22001390001.

Microbiome Biobanking: The missing link

Kostic, Tanja[1]; CONSORTIUM MICROBE[2]

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Culture collections have a history of supporting microbiological research, primarily through theaccession, preservation and supply of axenically cultured microorganisms. However, in nature,microorganisms do not exist on their own; they interact with millions of other microorganismsin complex systems called microbiomes [1]. Microbiomes provide various crucialecosystem services and are thus essential to the well-being of plants, animals and the environment.With developments in technology, microbiome research is changing the way culture collections and biobanks need to support their user communities. Key requirements for microbiome biobankinghave been proposed [2, 3], including the development of standards, and emphasising the need to deposit material and supply cultures, samples and associated data for futureresearch. Additionally, this will provide a mechanism to protect intellectual property, and helpresearchers adhere to legislative and regulatory requirements, including the Nagoya Protocol of the CBD. Integral to the above is the need to preserve environmental samples and their microbiota. MICROBE project (www.microbeproject.eu) aims to deliver innovative validated technological approaches that will enable:

- maximal preservation of taxonomic and functional biodiversity in selected microbiome samples;
- optimal collection and preservation of microbiome samples for defined subsequent analyses;
- targeted isolation of microbiome members from different domains and assembly of synthetic consortia that retain (and even optimize) the functional diversity of original microbiomes.

Furthermore, MICROBE will provide a comprehensive operational blueprint for the establishment of microbiome biobanking infrastructure, including technological requirements, methodological workflows, data pipelines, standards, legal and ethical guidelines, training plans and businessopportunities. First insights from the project will be provided.

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Acknowledgements

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Cryopreservation and recovery of a complex hypersaline microbial mat community

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Cryopreservation of microorganisms is an essential tool in industrial and food applications where conservation of microbial activity and critical beneficial traits need to be guaranteed to provide aconsistent product or production process. This often refers to simple, single species or low diversity assemblages in liquid cultures that can easily be revived and regrown to perform the desired process. Cryopreservation is also of essence for scientific experimentation where many environmental samples are taken in remote sampling sites and at high costs. Biobanking, or the long term preservation and potential revival of complex, structured samples come with an additional challenge related to maintaining the structure upon revival. Here we look at cryopreserving and reviving a complex photosynthesis driven microbial mat from a hypersaline ecosystem. Amplicon sequencing of the 16S and 18S ribosomal RNA gene was used to determine the community composition of bacteria and eukaryotes respectively. The tests included the use of different cryopreservative agents and different times of cryopreservation at 150 °C. Upon revival, the cryopreservatives cannot be separated from the preserved samples without disturbing the community structure, while carryover of these compounds may influence reconstitution of the communities. Indeed, although both glycerol and Me2SO are good cryopreservatives of microbial assemblages, carryover of these compounds had a profound negative effect on the reestablishment of a functional microbial mat. Best cryopreservation and reconstitution results were obtained in the absence of a cryopreservative agent or when methanol was used.

Direct Injection Mass Spectrometry for the Real-Time Volatilomics in Food System Microbiomes: the Potential of Providing Temporal Dimension in Multi-Omics Studies

Corvino, Antonia[1]; Capozzi, Vittorio[2]; Khomenko, Iuliia[1]; Biasioli, Franco[1]

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Fermentation exemplifies sustainable innovation in food systems by utilizing microbial diversity to enhance product quality and safety with low environmental impact. Diverse fermentation processes also serve as model ecosystems for microbiology studies and bio-tool design. Understanding and optimizing fermentation through 'omics' technologies, especially metabolomics, is crucial. Metabolomics offers insights into the metabolic state of microorganisms and the dynamics of metabolic pathways. Microbial Volatile Organic Compounds (mVOCs) are key metabolites that influence sensory perception and consumer acceptance. Profiling VOCs during fermentation provides valuable data on microbial ecosystems, identifying markers to monitor fermentation dynamics and optimize product yield, quality, and safety. Proton Transfer Reaction-Time-of-FlightMass Spectrometer (PTR-ToF-MS), a Direct-Injection Mass Spectrometric (DIMS) technology, enables non-destructive, real-time analysis with high automation and sensitivity. PTR-ToF-MS effectively evaluates microbial volatilome in various agro-industrial fermentation processes. We present case studies demonstrating PTR-ToF-MS's potential for volatilomics in food microbiologyand microbiome research, emphasizing the significance of temporal dimension metabolomics. This approach is particularly relevant for integrating the temporal perspective in multi-omics studies of dynamic systems like food-related microbiomes.

Acknowledgements
We developed this overview in the framework of the projects i) "Interconnected Nord-Est Innovation Ecosystem (iNEST) funded by the European Union Next-GenerationEU (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR) – MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.5 D.D. 1058 23/06/2022, ECS00000043) and ii) "ON Foods-Research and innovation network on food and nutrition Sustainability, Safety and Security– Working ON Foods" funded by NextGeneration EU [PNRR], in the framework of the Component 2 Investment 1.3Award Number: Project code PE00000003, Vittorio Capozzi is also supported by NUTRAGE CNR project FOE2021 DBA.AD005.225.

Possible “microbial bioremediators” isolated from wasted lands

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Human population is increasing every year and consequently the need for goods and services, and the associated waste and refuse. The current practices used for waste disposal usually cause considerable damages to environment, reducing the disponible land for agriculture and raw foodproduction; furthermore, the constant use of pesticides, phytochemicals and antibiotics to improve productivity is negatively impacting the consumers’ health. This research aims at proposing solutions which can improve the current situation of soil health with direct consequences on humanhealth by using selected microorganisms able to reduce harmful substances.

As a first part of this project, microbiological parameters were analysed starting from ten differentlands wasted by anthropic activity. The available results are as follows (average values): Pseudomonas spp. (9.40*106 CFU/g), actinobacteria (4.09*106 CFU/g), clostridia (1.35*102 CFU/g),and aerobic bacteria (3.53*106 CFU/g). Pseudomonadaceae were chosen and isolated because of their involvement, as demonstrated in other studies [1,2], in bioremediation activity against heavy metals and organic pollutants.

From a chemical perspective, the health profile of soils was studied, with a focus on heavy metals, for every soil sample and compared to the microbiological parameters to search clusters and correlations among the factors useful to deepen the genomic research.

Then, all the isolated strains were tested for phenotypic and technological parameters to assess the main characteristics with a focus on the resistance to heavy metals.

The information acquired in the first phase will be the background to assess the reducing capacityagainst heavy metals, the metagenomic research on soil samples and the 16S RNA analysis on selected strains.

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Food microbiomes and packaging: past, present and future

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Microbiomes are resources of growing interest for promoting sustainable innovation in food systems.Applications in the field of packaging represent an emerging sector that is still little explored. Here, we propose a review of the different types of packaging that include microorganisms and which are used/proposed for the food industry, giving particular emphasis to edible packaging containing lactic acid bacteria and packaging solutions that enhance microbial biocontrol activities. In light of the solutions that use single resources, a discussion is proposed on the potential use of microbiomes, carrying out a comparative analysis of the strengths and weaknesses. This is an extremely dynamic sector with broad prospects, crucial for the development of sustainable solutions for the food industry.

Acknowledgements
We are exploring the potential of this driver of innovation in the framework of the ongoing projects i) INTACTBioPack INTelligent, ACTive MicroBIOmebased, biodegradable PACKaging for Mediterranean food – founded by the PRIMA Section 2 2023 multi-topic and ii) the NextGeneration EU [PNRR], in the framework of the Mission 4 Component 2 Investment 1.3-Award Number: Project code PE00000003, Project title: "ON Foods-Research and innovation network on food and nutrition Sustainability, Safety and Security–Working ON Foods".

Marine microbial community sample cryopreservation: finding the best preservation strategy

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Marine microbiomes comprise communities of diverse microorganisms (bacteria, archaea, protists and microalgae) and their 'theatre of activity' (i.e. metabolites, signalling molecules and surrounding environmental conditions). These microorganisms play an essential role for the oceans and our planet by participating in the balance of natural cycles (nutrients, oxygen and carbon dioxide). In order to facilitate the science required to make major advances in microbiome research, methodsand technologies are needed to capture and ensure stable long-term maintenance. Since 2010, the Roscoff Culture Collection (RCC) has cryopreserved more than 3000 mono-specific phototrophic marine microbial strains, including different lineages of microalgae and cyanobacteria. The RCCis now developing methods to cryopreserve complex marine microbiomes with the aim of capturing and culturing the widest possible range of microbial diversity. We investigated the effects of different cryoprotectants on the viability of coastal marine microbiomes using various approaches to assess post-cryopreservation viability. Once samples were thawed, we tracked changes in the microbial community using RNA metabarcoding, measured growth rates using cytometry, and useda variety of traditional methods to culture bacterial and algal strains. Together, the results will be used to identify the best strategy for conserving marine microbial community samples through cryopreservation.

Fermenting metacommunities of food interest: an invaluable biodiversity asset to be maintained overtime in its wholeness and functionality Micro4ever

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Micro4ever aims to investigate the effect of cryopreservation (CrP) and freeze-drying (FD) onthe functionalities of whole microbial consortia (MC) associated with different fermented foods (dairy and bakery products) after their long-term storage and revitalization. Specifically, we will use the MC of milk starters (lattoinnesto; LI), whey starters (sieroinnesto; SI) and sourdoughs (SD) as models of dynamic MC characterized by a high level of biodiversity. The main objectives of Micro4ever are: (i) to preserve intact MC with retained viability and functionality for future meta-omics studies, cultivation, and application; (ii) to preserve and exploit in situ the invaluable biotechnological potential behind the poorly cultivable microbial players of MC; (iii) to optimize the conditions of sampling and long-term storage of MC, providing the maximum protection to different kinds of cells and cellular components within the MC; (iv) to validate the post-preservationviability and functionality of preserved MC; (v) to propose new models governing the assembly, organization, functionality and stability of MC of food interest. While the advantages of fully exploiting the whole functional potential of MC are clear, the accomplishment of that intent is fraught with conceptual and technical challenges. Micro4ever will establish and optimize the cultivation and preservation methods that protect the full phenome of MC and ensure genotypic stability of the MC, which is essential for the preservation of MC biodiversity. In particular, Micro4ever will implement a multi-omics workflow, through which we will first decipher the taxon composition and the functional redundancy of MC from LI, SI, and SD at metagenomics, metatranscriptomics,meta-phenomics and metabolomics levels. Then, we will investigate in depth the effect of short- and long-term storage, and will validate the effectiveness of CrP and FD protocols and the functionality of preserved MC also in food matrices.

Acknowledgements
Project funded by the European Union Next Generation EU; Project code P2022RJYCN, CUP H53D23010720001, Project title "Fermenting metacommunities of food interest: an invaluable biodiversity asset to be maintained overtime in its wholeness and functionality Micro4ever".

Biochemical and biophysical characterization of two recombinant enzymes obtained from termite gut metagenome

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In this study, we characterized two novel glycoside hydrolase family 10 (GH10) enzymes (Xyl10C and Xyl10E) [1] identified in the termite gut microbiome. Both enzymes showed activity on beechwood xylan, whereas Xyl10E also showed activity on barley -glucan. Purified Xyl10C showed optimum activity in the pH range 7.0-8.0 and at a temperature of 50-60°C, while Xyl10E was active at a wider pH range (5.0-10.0), and at 50°C. The residual activities of Xyl10C and Xyl10E after 8 hours of incubation at 40°C were 85% and 70%, respectively, showing thermal stability. The enzymatic activity of Xyl10C increased in the presence of 5 M NaCl (115%) and was only inhibited in the presence of 0.5% SDS and reduced with β-mercaptoethanol, whereas both the xylanase and glucanase activities of Xyl10E were inhibited only in the presence of $MnSO_4$, NaCl and SDS. In addition, both enzymes were able to hydrolyze pretreated Sorghum bicolor bagasse (SBB), releasing xylose and xylobiose as the main products [2]. Xylose and xylobiose productionssuggest their potential use as prebiotics and as other valuable industrial applications.

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Acknowledgements
This study was supported by grants from (INTA) (PI 085, 089, 122 and 159), the Agencia Nacionalde Promoción Científica y Tecnológica (ANPCyT) Proyectos de Investigación Científica y Tecnológica (PICT) 2018-#4149, 2019-#3156, 2020-#3570, Fundación Williams #014 and CONICETProject # PIP-2021-2561. The authors thank the MICS (Made in Italy—Circular and Sustainable) Extended Partnership from the European Union Next-Generation EU (PIANO NAZIONALEDI RIPRESA E RESILIENZA (PNRR) —MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.3—D.D. 1551.11-10-2022, PE00000004).

Exploring Microbiomes in PFAS Contaminated Environments: Preservation for Bioremediation Strategies.

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Per- and poly-fluoroalkyl substances (PFASs) are a group of xenobiotics widely used for their water and grease-resistant properties [1] that have raised significant environmental and health concerns due to their persistence and potential toxicity [2]. Their bioaccumulation led to biomagnification upthe food chain, exerting their toxic effects, and increasing the likelihood of severe health outcomesover time [3]. Bioremediation may be a promising strategy to mitigate PFAS effects in contaminated environments. Understanding microbiomes in PFAS-polluted sites is crucial due to their potential for bio-transforming or bio-accumulating PFAS. Thus, our focus is to study microbiomesin PFAS-polluted environmental matrices and their potential for PFAS bioremediation. This aimis pursued in conjunction with the SUS-MIRRI.IT project regarding the study of microbiome in different matrices. The experimental approach involves preserving environmental samples to study how microbial communities can be efficiently stored preserving their bioremediation potential. To achieve our objectives, we analyzed two different matrices: soil and sediment. Soil samples (microbiomes) were preserved at -80°C (for 12 months) in glycerol (30%) as a cryoprotective agent to investigate their vitality through total microbial counts and Ecoplates assays. Additionally, we employed microbial barcoding to identify microbial diversity and the bioremediation potential ofthese microbiomes will be evaluated through PFAS content determination. We decided to perform these analyses over a twelve-month time span; T0 (pre-storage), T1 (3 months post-storage), T2 (9 months post-storage), and T3 (12 months post-storage). Preliminary results (T0 and T1) showsuccessful preservation. However, confirmation awaits experiments at T2 and T3. Further analysis at T2 and T3 will be performed to determine the long-term effects of preservation of samples on the microbiome and their potential application to bio-transform PFAS.

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UMCC's investigative approach for the preservation and exploitation of fermented beverage microbiomes

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The role of microbial culture collections is fundamental for the preservation, study and utilization of microbial resources. Although the conservation and maintenance of pure microbial strains is a well-established procedure within the management system of microbial collections, the preservation of microbiome, defined as the entire microbial community that occupies a defined habitat and their theater of metabolic activities [1], still needs to be consolidated as a routine practice. However, acquiring knowledge on microbiome conservation and a better understanding of the interactions among microbial communities and food substrates, is essential for their biotechnological valorization.
Unimore Microbial Culture Collection (UMCC) (https://umcc.bioaware.com/), located at the University of Modena and Reggio Emilia, cryopreserves over 3000 microbial strains isolated from different food substrates. Recently, UMCC began an in-depth study regarding microbiome preservation from fermented beverages. A total of ten microbiome samples from wine vinegar, pomegranate vinegar, apple cider vinegar and Kombucha tea were appropriately sampled, analyzed through both culture-dependent and molecular approaches, and preserved by ultrafreezing and freeze-drying. A comparative evaluation of microbiomes before and after storage is currently underway with the aim of evaluating the effectiveness of long-term storage methods and, then, the possible industrial exploitation of microbiomes.

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Acknowledgements
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A cryopreservation protocol of microbial consortia from Apulian table olives

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The field of microbiome research has evolved rapidly over the past few decades and has become atopic of great scientific, industrial, and public interest. Exploiting microbiomes as drivers of innovation in food systems and promoting reproducibility in basic, applied and industrial science shed new light on the preservation of microbial communities associated with food fermentation/specific matrices. Therefore, the challenges of optimally preserving microbiome samples are huge, with difficulties ranging from conserving microbial consortia to preserving microbiome integrity/functionality. Fermented table olives represent the most representative and diffused plant-based fermented products in Mediterranean countries, with relevant productions in Italy, in general, and in the Apulianregion, in particular. Within the SUS-MIRRI.IT project, a cryopreservation protocol has been developed to preserve the microbiota from typical Apulian table olives cv Leccino, by using glycerolor DMSO as cryoprotectants, and a storage temperature of -135 °C. The microbial consortium was studied before and after mid-term storage using a culture-dependent approach, RNA-based metabarcoding analysis, and metabolic profiling evaluation. Results showed that after six monthsof cryopreservation, the viability of the microbial consortium slightly decreased regardless of the cryoprotectant used, and no significant changes in the metabolic profile were observed. Also, the metabarcoding analysis showed no significant differences in relative abundances after storing period. Results confirmed the effectiveness of the developed cryopreservation protocol, proper preservation of the microbial consortium, and its functionality during a mid-term storage period.They also pointed out preliminary insights on the possibility of exploiting them in fermenting processes after cryopreservation.

Acknowledgements
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Synthetic microbial communities as a tool to understand microbial interactions and their role in bacterial cellulose production

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Genetic and physiological diversities among microorganisms result in the emergence of complex microbial communities, where interactions among cells can influence each other's behaviours and phenotypes. Microbial communities play a crucial role in the functionality of several ecosystems,such as bio-geochemical cycles, agri-food productions, and human health, boasting an enormous metabolic potential. The construction of synthetic microbial communities allows the generation of targeted systems with reduced complexity, capable of achieving multiple tasks in a more efficient way compared to single strains. Synthetic microbial communities may be exploited as models to understand microbial dynamics and regulatory pathways, as well as tools for several biotechnological applications in chemical, pharmaceutical, and agri-food fields. Within the PRIN 2022 Project "BioCellulose production from a Synthetic Microbial Community: sustainable process for food and healthy applications SynBioCell" (code: 20228Z34PF, CUP: E53D23010680006), a novel synthetic microbial community is being designed and assembled. Following a chemometric approach, different species belonging to acetic acid bacteria, lactic acid bacteria and yeasts were tested for their suitability to be combined in a synthetic microbial community, aimed at enhancing bacterial cellulose synthesis using various defined media and agri-food substrates. Each synthetic microbial community member will cooperate for the development of a functional ecosystem, where interactions and metabolite sharing are believed to promote cell growth and bacterial cellulose yield. The development of a targeted synthetic microbial community may help facing the increasing demand of bacterial cellulose and other bioproducts through a sustainable and efficient approach.

Acknowledgements
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Back-slopping of dried, freeze-dried and frozen Tempeh: the effect of long-term preservation techniques on the microbiome and volatilome

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Tempeh is a traditional Indonesian fermented food, commonly made from soya, but often also from peas, beans, chickpeas and other legumes. It is mainly produced in Asia, particularly in Indonesia,where it is a staple food, but its production is also growing in India, Japan, United States and Europe. Tempeh is an ideal meat substitute, high in protein, and a nutritious food that may conferhealth benefits due to its high bioavailability of nutrients and phytochemicals, showing improvedaction on oxidative stress, glycaemic control and blood lipid levels [1].
Tempeh production involves several steps, including soaking, dehulling, cooking and fermentation with the starter mould *Rhizopus* spp. However, the nature of the tempeh production processes creates a unique microbiome derived not only from the microbial inoculum, but also from the raw materials and environment. Over the past decade, metagenomic studies of the microbial community during tempeh production highlighted a complex microbial consortium consisting not only of *Rhizopus* spp. (used as inoculum) but also of other microorganisms belonging to the phyla Proteobacteria, Firmicutes, and Bacteroidetes [2].
Preservation of the tempeh microbiome is essential to maintain its functional and nutritional properties. This can be achieved through proper fermentation processes, storage, and handling techniques to minimize contamination and degradation of the microbial community [3].
Our aim was to evaluate the effect of different long-term preservation methods (drying, freeze-drying and freezing) on the tempeh microbiome and the possibility of reusing the stored productas a starter for a subsequent back-slopping fermentation, a method in which a portion of the fermented product is added back to the new batch to continue fermentation. Tempeh stability over time was evaluated for microbiome and volatilome (HS-SPME-GC-MS) aspects.

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New frontiers for *Saccharomyces cerevisiae* flor strains

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The process of biological ageing by *Saccharomyces cerevisiae* flor strains characterizes wines of considerable value, such as Spanish Sherry wines and some traditional wines of Sardinia such as Malvasia di Bosa, Vernaccia di Oristano, and Arvisionadu del Goceano. This process is dependenton FLO11 gene expression and involves the transition from fermentative to oxidative metabolism,with the formation of the typical yeast biofilm (flor) on the wine surface at the end of fermentation. In recent years, the evolution and application of modern winemaking techniques, which are increasingly effective in avoiding the presence of even minimal oxygen concentrations during wine ageing, makes it more difficult to isolate flor yeasts from the Sardinian wines. In this context, the role of culture collections for the supply and valorization of microbial resources previously isolated and characterized, appears crucial. In Sardinia, the MBDS-UNISSCC microbial collection (certified ISO 9001:2015) belonging to the MIRRI-IT network of collections and equipped with a federated database "www.mbds.it", preserves microbial resources isolated from food and environmental matrices. Yeast strains, including flor strains, of oenological interest have been isolated from Sardinian musts and wines, characterized, and stored since 1960. Currently, these resources are cryopreserved at -80 °C and have shown a satisfactory response during viability testing. Recently, the flor formation ability of 56 strains ascribed to the genus *Saccharomyces*, isolated, characterized and stored between 1965 and 1985, has been confirmed. MBDS-UNISSCC aims to preserve this microbial biodiversity by characterizing all the stored flor strains at the molecular level and developing preservation methods of the whole biofilm.

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Microbiome characterisation of sourdough from the MBDS-UNISSCC microbial collection

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The MBDS-UNISSCC includes a collection of over 80 sourdoughs originated from different areasof Sardinia and various types of bread. Three of these sourdoughs, namely SD81, SD82, and SD84, have been chemically, microbiologically and technologically characterized, duplicated and stored at -80 °C to assess the stability of the microbial consortium during and after storage. Each sample underwent microbiological analyses using standardized procedures, both for sampling and DNA extraction. As a physiological test, the microbiomes from the three samples were evaluated for their ability to metabolize over ninety carbon and nitrogen sources using OmniLog® PM technology. In samples SD82 and SD81, the bacterial populations prevailed over yeasts, while in SD84, the abundances were similar. In SD81, the most represented bacterial isolates belonged to *Fructilactobacillus sanfranciscensis*, followed by *Levilactobacillus brevis* and *Lactiplantibacillus plantarum*, while the prevalent yeast species were *Kazachstania pseudohumilis*, *Pichia kudriavzevi*, and *Torulaspora delbrueckii*. In SD82, all the bacteria (24 isolates) were identified as *F. sanfranciscensis*, while the yeasts belonged to Kazachstania humilis and Saccharomyces cerevisiae. SD84 shows higher biodiversity with *Pediococcus parvulus* and *P. kudriavzevi* as the predominant bacterial and yeast species, respectively. The SD81 microbiome was capable of catabolize 43% and 88.5% of the tested carbon and nitrogen source, showing a high phenotypic diversity compared to SD84and SD82. In comparison, SD84 and SD82 microbiomes were only able to catabolise 33.4% and 29% of nitrogen sources and 38% and 8% of carbon sources, respectively. Overall, the microbiomes of samples 81 and 84 showed greater phenotypic diversity compared to that of SD82. Long-term preservation strategies based on the extraction and storage of the microbiome from the sourdoughs are currently being implemented within the SUS-MIRRI project.

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Culturomics and microbiome-based approach for the development of NatComs as biofertilizers and biocontrol agents for sustainable agriculture: the reinforcement of ENEA microbial culture collection.

Sbarra, Federico[1,2]; Visca, Andrea[1]; Sevi, Filippo[1]; Tabacchioni, Silvia[1]; Colantoni, Eleonora[1]; Di Gregorio, Luciana[1]; Costanzo, Manuela[1]; Aracri, Benedetto[1]; Del Fiore, Antonella[1]; Varese, Giovanna Cristina[2]; Bevivino, Annamaria[1]

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Beneficial microorganisms can be used to increase crop yield and/or to reduce the damage caused by pathogens and pests in agricultural fields. Synthetic microbial communities (SynComs) failto mimic the natural composition, the diversity and the ecological functions found in the original ecosystem. In the present work, a combined culturomics and metagenomics approach was explored to define the core microbiome of two composite rhizosphere samples from strawberry (*Fragaria* spp.) plants grown in field and to identify strains with plant-growth promoting (PGP) and biocontrol activity for the set-up of tailored Natural microbial communities (NatComs). Thetotal DNA of the microbial community was extracted and used as template for PCR targeting theregion ITS1-ITS4 for eukaryotic samples and V3-V4 for prokaryotic samples. The raw sequenceswere filtered and denoised and the ASV were taxonomical assigned using Silva 138.1 database. Microbial community growth strategy was investigated by means of total microbial count, enumeration of copiotrophs and oligotrophs and eco-physiological (EP) index. A total of 159 bacteria, 57 putative N-fixing bacteria and 14 fungi with different morphologies were isolated. Bacterial isolates were characterised for siderophore production, using chrome azurol S (CAS) agar plates, and in-vitro antifungal activity. A total of 109 strains were identified as siderophore producers based on the halo thickness around the colonies. Only one fungal strain resulted positive to P-solubilisation assay, using Pikovskayas medium, and nine strains resulted positive to the PCR amplification of nifH gene. Overall, a total of 119 out of 227 isolates with PGP activities were selected and will be identified by 16S/ITS sequencing and MALDI-TOF technology. The well-characterised strains will be included in the database of ENEA Microbial culture collection and exploited for the set-up of NatComs.

Acknowledgements
The authors thank Luca Cocolin for his valuable support, and Davide Spadaro, Marco Garello and Francesco Aloi for providing rhizosphere samples. This activity has received funding from the SUS-MIRRI.IT Project "Strengthening the MIRRI Italian Research Infrastructure for SustainableBioscience and Bioeconomy", Project code IR0000005, CUP D13C22001390001.

Trichoderma asperellum and *Bacillus* spp. antifungal activity evaluation against *Fusarium* fungi

Seņkovs, Maris[1]; Jauga, Kristians[2]; Nikolajeva, Vizma[3]; Petriņa, Zaiga[1]

Researcher[1]; student[2]; associate professor[3]

The aim of the study was to investigate the antifungal activity of *Trichoderma asperellum* MSCL 309 as well as *B. subtilis* MSCL 897, *B. subtilis* MSCL 1441 and *Priestia* (*Bacillus*) *megaterium* MSCL 49 against twelve phytopathogenic *Fusarium* spp.. Fusarium isolates previously isolated from oats was evaluated in three independent experiments involving double culture, volatile anddissolved compound detection methods. In a fourth experiment, the effect of *T. asperellum* solutes on the growth of Fusarium isolates was tested. In the double culture method, *T. asperellum* inhibited Fusarium strains AS 181401 and AS 1809025 most actively, and AS 070701 strain least actively. The highest antifungal activity of *T. asperellum* liquids was found against *Fusarium* AS 181202S, *Fusarium* AS 181501S, *Fusarium* AS 181401S, *Fusarium* AS 70701, *Fusarium* AS 071301S and, Fusarium AS 70402 isolates. No significant effect of volatiles from *T. asperellum* strain on any *Fusarium* isolate was detected. *B. subtilis* strain MSCL 1441 inhibited the growth of all *Fusarium* spp. isolates, while *B. subtilis* MSCL 897 and *P. megaterium* did not inhibit the growth of any *Fusarium* isolates.

The use of microbial inoculants for the control of Fusarium related diseases in durum wheat

Alberoni, Daniele[1]; Cappelletti, Eleonora[1]; Cali, Martina[1]; Amaral Carneiro, Greice[1]; Rabbi, Francesca[1]; Prodi, Antonio[1]; Di Gioia, Diana[1]

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Biocontrol to counteract pathogens in agricultural crops with the use of selected microorganismsrepresents a sustainable alternative to pesticides. Durum wheat, a significant cereal crop globally, faces threats from diseases like *Fusarium* Foot Rot (FFR), *Fusarium* Crown Rot (FCR), and *Fusarium* Head Blight (FHB). These diseases, induced mainly by *Fusarium culmorum*, *Fusarium graminearum*, and *Fusarium pseudograminearum*, cause yield and quality losses [1]. Current farming defenses rely on agronomic practices and fungicides. However, due to resistance phenomena, environmental impact and toxicity towards consumers, greener alternatives are looked for. In this work, about 90 bacterial strains, 75 of which isolated from durum wheat, were screened in vitro against *Fusarium* strains using different microbiological assays. Promising candidates mainly belonging to the *Bacillus* and *Pseudomonas* genera and Lactic Acid Bacteria (LAB) were selected for further studies. In vitro seed priming using the selected strains of treatedseeds proved effective against FCR/FFR with also positive influence on the germination capacity.In controlled environment trials, a LAB strain was able to *F. culmorum* at crown level, stimulatingplant growth. Open-field trials treatments with the selected microorganisms exhibited positive effects in FHB control. Metabolomic profiling underscored the presence of antifungal compounds, showing the synthesis of surfactin and fengycin by *Bacillus* species, whereas benzoic acid and hydrocinnamic acid were identified in *Lactobacillus* strains. Overall, this biocontrol approach provides valuable insights into the applications of beneficial bacteria for managing Fusarium-related diseases in durum wheat.

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Acknowledgements

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Session 6 – Microbes and Citizen Sciences: through the lens of public opinion

Thursday 19th September, 4:00pm

Chairs: Moretti Antonio (Italy), Perrone Giancarlo (Italy).

The Isala project: characterizing the female microbiome through citizen science

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The composition of the vaginal microbiome is known to be associated with several aspects of women's health and reproduction. Studies on the vaginal microbiome have until recently been mostly conducted in a clinical setting, which limits attainable sample sizes and which may createvarious biases in the sample. One way to overcome these limitations is a citizen science approach,where participants take their own samples in the comfort of their home. However, such an approach comes with its own set of challenges concerning recruitment, logistics, quality control and communication. In 2020, we launched Isala: a citizen science project aiming to study the female microbiome of women from the general population of Flanders, Belgium. For the first phase of the project, participants were asked to take swabs from their vagina and some other body sites and fill in a very extensive questionnaire. Recruitment was handled by launching a general call through social and regular media channels and by framing the project in a larger societal context surrounding women's health. A web designer and a graphic design firm were hired to create the sampling kits, promotion material and a professional website. Samples were transported through the Belgian postal office, after a pilot study showed no adverse effects on sample quality. The highlights of the results were communicated through various Belgian media, while an abstracted version of participant's personal microbial profiles were sent to them through the website. After the initial call for participants, more than 6,000 women registered in a single week, while quality control of the microbiome samples indicated a very high overall sample quality. The data has already led to various insights on the vaginal microbiome of women in Flanders, associations with lifestyle and other factors and co-occurrences between vaginal taxa. Follow-up projects are currently underway and are yielding more specific insights.

The Isala project was created by a team of researchers led by professor Sarah Lebeer at the University of Antwerp and was also made possible by some academic and non-academic partners. All are listed at https://isala.be/en/about/.

Mediterranean Diet and microbes: an emerging target for educational paths and citizen science

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The Mediterranean Diet represents a dietary model, but more generally, a set of good lifestyle practices, which receives particular attention for its impact on well-being in childhood, adult life and ageing. While the diversity of foods of plant origin (i.e. legumes, vegetables) are generally well correlated with the strengths of the Mediterranean Diet, little emphasis has usually been given to aspects relating to microbial resources, i.e. fermented foods, starter cultures, and dietary microbes. The intake of fermented foods, bio-molecules of microbial origin and microbes in the diet represent an area that is receiving growing interest in various fields of science. Here, we present an overview of Mediterranean fermented products, highlighting the interesting aspects of the main categories. Regarding the categories, recent works are presented that promote knowledge in food microbiology as topics to promote educational paths at school and university levels, as well as citizen science experiences. Finally, the experience carried out in schools is reported, underlining how the microbiological aspects represent a topic that allows the Mediterranean Diet to be promoted in a new light to students and citizens.

Acknowledgements
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 within 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 funded by CNR project “NUTRAGE FOE2021 DBA.AD005.225". Mariagiovanna Fragasso is supported by the European Union NextGeneration EU founded ‘Agritech National Research Centre for Agricultural Technologies’ [PNRR, CN00000022]

Didactic Proposals in Education for Sustainable Development in Teaching in Portugal and Brazil: Circular Relationship between Climate Change, Healthy Ecosystems and Soil Microbial Diversity

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Several educational materials, when explored in a formal or non-formal teaching milieu and mediated by a teacher or educator, become rich in meaning for the students. The co-creation of new teaching materials for Education for Sustainable Development (EDS) is, therefore, a permanent need that requires constant updating. For this study, we consider climate change, healthy ecosystems and soil microbial diversity, as societal challenges to be addressed by EDS. Additionally, anthropogenic pressure on Earth has reached an unprecedented scale, where abrupt global environmental changes can no longer be ignored. In this context, the study starts from the following research question: How to create didactic proposals for Education for Sustainable Development in children's Education in Portugal and Brazil which facilitate a circular view of the relationships between climate change, healthy ecosystems and soil microbial diversity? The methodology of this study is qualitative and comparative, based on content analysis (curriculum official programmes and textbooks) and their gaps. The first preliminary results of this analysis will be presented andshow how the role of microorganisms is almost absent from the children's teaching contents. Based on the results, it subsequently, proposed that the study will be complemented with semi-structured interviews with teachers from early grade levels of education in Portugal and Brazil, to end with the co-creation of proposals for teaching activities, including manipulatives, and outdoor activities, among others, and which will be validated by the interviewed teachers. The outcome of this PhD thesis project is expected to obtain didactic proposals for children in the context of formal or non-formal education, for sustainable development.

Acknowledgements
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Session 7 – Advanced approaches in taxonomy, phylogeny and functional genomics

Friday 20th September, 9:30am

Chairs: Clermont Dominique (France), Masiello Mario (Italy).

Metabarcoding with Nanopore MinION: not only 16S or ITS

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The microbial species identified so far account for only a small portion of Earth's overall biodiversity. To enhance our understanding, DNA barcoding serves as a powerful tool for identifying multiple species, relying on the amplification and sequencing of conserved genetic regions. However, traditional short-read sequencing offers limited taxonomic resolution due to incomplete sequences. The advent of new long-read sequencing techniques, such as Nanopore sequencing, addresses this issue. Long-read sequencing improves species identification accuracy and increases the ability to detect low-abundance species by including larger portions of the standardized marker sequence. Despite these advances, there is a shortage of bioinformatics tools specifically designed for analyzing Nanopore sequence data compared to earlier technologies. Consequently, our study focused on establishing an amplicon-Nanopore sequencing workflow and developing a pipeline capable of processing long, noisy reads. To evaluate and compare our pipeline with existing methods, we amplified and sequenced the 16S and ITS genes of various known microorganisms with differing taxonomic similarities using Nanopore technology. The resulting sequences were analyzed using both our pipeline and available software. Our results demonstrated precision and recall rates close to 1, comparable to the Emu software. Additionally, we tested the flexibility and reliability of our method by applying it to other selected genetic markers to achieve detailed identification at the pathovar/subspecies level for quarantine bacterial pathogens. Our approach proved promising for the precise identification of microorganisms at the genus-species level for broader metabarcoding studies and deeper taxonomic resolution, such as pathovar or sequence type.

Genomic overview over 100 cyanobacterial strains: taxonomy, microbiome and biosynthetic gene clusters

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In the Bank of Algae and Cyanobacteria of the Azores, there are more than 800 non-axenic strainsof microalgae and cyanobacteria, and more than 400 correspond to cyanobacterial strains. In the last year, five genera and ten species have been described based on the polyphasic approach increasing the available data for genetic studies, but lacking genomic data. To better support further taxonomic studies, more than 100 non-axenic strains have been sequenced by Illumina. Most of the cyanobacterial genomes produced are of high quality with >99% completeness and
<1% contamination. Although the objective of this study was the cyanobacterial genomes, many bacteria associated with the cyanobacterial cultures were also sequenced, providing high-quality genomic data and allowing the characterization of the microbiome associated with the cyanobacterial organism. This data allows for higher quality taxonomic studies of cyanobacteria in several problematic clades, such as the *Prochlorococcaceae*, and complements biased single-gene phylogenies. It can also support targeted studies by taking into account genomic analysis and the diversity of biosynthetic gene clusters (BGCs), not only of the cyanobacteria but also by the associated microbiome with novel genomic information. The genomic study of non-axenic cyanobacterial strains can provide many benefits, not only by studying the target organism but also the associated microbiome, which is likely to contain many unculturable bacteria that represent unique resources for taxonomic, conservation and biotechnological studies.

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Drivers of aquatic fungal diversity and ecological functions throughout Europe: Biodiversa+ FUNACTION and MoSTFun projects

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Aquatic fungi (AF) and fungal-like organisms are ecologically diverse, play key functions in freshwater and marine ecosystems and provide numerous ecosystem services and disservices. Despite their relevance, quantitative evidence about AF diversity and functions is still scarce. The panEuropean FunAqua project, led by our Estonian team, implemented eDNA metabarcoding to explore the taxonomic, phylogenetic, and functional diversity of AF at the global scale. Capitalizing on this experience, we developed two transnational projects within the Biodiversa+: FUNACTION and MoSTFun.

FUNACTION aims to fill knowledge gaps in AF ecology and develop conservation guidelines basedon AF diversity recorded across Europe. FUNACTION will implement a modeling approach tomap drivers of AF diversity change and identify priority areas for conservation within the EUBiodiversity Strategy for 2030. Members of the project, together with worldwide AF experts, alsoinitiated the Aquatic Fungi Specialist Group of the IUCN Species Survival Commission (SSC),aimed at coordinating the first conservation assessment, planning and action efforts for AF. MoSTFun aims to integrate AF into practicable monitoring procedures based on Essential Biodiversity Variables and open accessibility of sequencing and environmental data in FAIR databases.MoSTFun will test novel approaches including -omics technologies, Earth Observation, GIS, andspecies distribution modeling leveraging on existing biomonitoring programs and networks to reanalyze samples and evaluate procedures to include AF in routine biodiversity monitoring.

Both projects, by harmonizing data and expertise on AF with existing initiatives, will set the stagefor broad exchanges with relevant stakeholders. This is a fundamental step to change people's perception about this neglected, yet highly diverse group of organisms and facilitate initiatives to preserve healthy aquatic ecosystems for future generations.

ITSoneDB v1.144 and BioMaS@ITSoneWB: two ELIXIR-IT main resources for amplicon based mycobiome investigation

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DNA-metabarcoding represents the cheaper and faster way to explore and profile the compositionof microbial communities colonizing several habitats, such as soil and water, as well as animal hosts niches as human skin and livestock guts. Profiling the microbiota taxonomic composition iscrucial for understanding how microorganisms may influence the overall state of the investigatedhabitats.
To date, the exploration of the so called mycobiota (i.e. fungal components of microbiota) is a constantly growing field and ITS1 metabarcoding is one of the most reliable approach [1]. The choice of the most appropriate bioinformatic workflow is a key point to obtain reliableand interpretable results.
In this scenario, we present ITSoneDB v1.144 [2] an ITS1 sequence databaseand BioMaS at ITSoneWB [3] a bioinformatic resource for Illumina paired endreads sequence analysis and taxonomic classification. We present a a comparative assessment of ITSoneDB versus UNITE [4] as well as of BioMaS@ITSoneWB versus QIIME2 [5]. Shortly QIIME2 relies on the inference of Amplicon Sequence Variants (ASV) by wrapping DADA2 algorithm, while BioMaS [6] is an ASV and Operational Taxonomic Unit (OTU) free approach particularly suitable for barcodes with a highly variable lengthas ITS1 (i.e. 100-1,000 nucleotides long).
The results of the comparative assessment, carried out on a known microbial mock community, will be presented providing evidences of the relative performances of the tested combinations of reference databases (ITSoneDB and UNITE) and classification tools (BioMaS and QIIME2).

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The new challenge in virus taxonomy: a binomial nomenclature for virus species

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The International Committee on Taxonomy of Viruses (ICTV) is responsible for developing and maintaining an internationally agreed system of hierarchical classification of viruses and naming of taxa, available to the scientific community through a website (https://ictv.global/) and publishedreports/papers. Over the years the ICTV has adapted its taxonomic framework to reflect current knowledge on the evolution of global virosphere. The ICTV now allows the classification of virusesknown only from genomic data. Taxonomic ranks have been expanded beyond orders to 15 hierarchical ranks, paving the way to comprehensive studies on evolutionary connections of virusesand bringing virus taxonomy closer to other biological taxonomies. Species naming in all these taxonomies follow a Latinized binomial format (i.e., binomial nomenclature) first introduced by Carl Linnaeus in 1753, consisting of two italicized words indicating the genus ("genus name") andthe species ("specific name/species epithet"), respectively. Typical examples of binomial species names are *Arabidopsis thaliana*, *Saccharomyces cerevisiae*, *Homo sapiens*, or *Escherichia coli*. On the contrary, a uniform format was not enforced for viral species names, which overlapped those of viruses, except for a requirement to be italicized and to have the first letter of the first word capitalized. To adopt a standardized format, a binomial nomenclature for virus species was ratified by the ICTV in 2021. Thus, a virus species name consists of two italicized words, the first one being the genus name and the second one being a "free-form" species epithet, composed using the standard Latin-script English alphabet containing 26 letters and/or Arabic numbers. Linnaean-style, Latinized virus species are permitted, but not mandated. Importantly, this change in nomenclature applies to virus species only. Established species names have all been converted to binomials.Common virus names are not affected and will stay unchanged.

Genomic insights into *Mrakia*: expanding horizons in microbial biotechnology

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In recent years, multi-omic sciences and next-generation sequencing (NGS) technologies have revolutionized microbiological research and microbial ecology, making DNA sequencing faster and more affordable [1]. This has enabled the examination of various ecosystems and the development of genetic tools for non-conventional yeasts (NCY), though the field is still in its early stages [2].

This study aims to define an efficient genomic analysis pipeline for NCY, non-model organisms, with a focus on the *Mrakia* genus, comprising 30 species belonging to the phylum Basidiomycota and of environmental origin. Interestingly, some species/strains display fermentation abilities suggesting biotechnological potential in fermented beverages, evidenced by successful tests on *Mrakia* gelida in beer prototype production [3,4].

In this context, producing high-quality genomic and transcriptomic sequences for different *Mrakia* species allows for a more comprehensive understanding of their genetic and functional bases, thus uncovering hidden potential in the fermentation sector and beyond.

Currently, aiming to sequence all *Mrakia* type strains, we obtained Oxford Nanopore long reads and Illumina short reads for 7 accessible species. To obtain high-quality genome assemblies, we tested 3 assembly and correction tools, selecting the best combination based on the assembly statistics. Additionally, repetitive sequence masking was performed. The average nucleotide identity between species genomes was calculated to assess phylogenetic relationships. Gene prediction will be conducted on the assembled genomes by means of transcriptome data to identify biotechnological fermentation markers.

Future plans involve broadening genomic and transcriptomic data to include 11 more *Mrakia* species, improving gene annotation, and enhancing taxonomic analysis. In conclusion, this studyaims to deepen our understanding of unexplored *Mrakia* species, maximizing their potential in microbiological and biotechnological fields.

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New Enterobacterales species isolated from dead seals in Antarctica

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One of the goals of Antarctic research is to monitor and preserve microbiological diversity in animals and the environment.
Among samples collected on James Ross Island within the Czech Antarctic Research Programme,two strains isolated from dead seals, P4252 and P6696, were not identified as known species using phenotyping methods and, therefore, chosen for further molecular analysis. These two strains were gram-negative, rod-shaped, fermenting, psychrotolerant, and moderately halotolerant bacteria.
Sequencing of the 16S rRNA gene revealed that the closest hits for strain P4252 were *Rahnella inusitata* with 98.41 % sequence similarity and *Rouxiella badensis* with 98.32 % similarity. The gene sequence of strain P6696 showed the highest similarity to *Moellerella wisconsensis* with 98.58% and *Providencia burhodogranariea* with 98.08 %.
Next, whole-genome sequences were obtained, and the Digital DNA-DNA Hybridization (dDDH)analysis was performed using the TYGS pipeline by DSMZ. The dDDH values were 23.6 % for *Ewingella americana* as the closest to strain P4252, and 22.7 % for *M. wisconsensis* as the nearestto strain P6696. These values are below the accepted threshold indicating that the studied strainsbelong to potential new species of the order Enterobacterales. The Average Nucleotide Identity (ANI) values were 81.08 % between strain P4252 and *E. americana*, and 76.44 % for strain P6696and *M. wisconsensis* which implies that the isolates represent novel species within one-species genera of *Ewingella* and *Moellerella*.
The analyses based on whole-genome sequences are more robust, thus the results of dDDH and ANI are more reliable than comparing the similarities of the 16S rRNA gene only.

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Taxonomic classification of Metagenome-Assembled Genomes with kMetaShot

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The investigation of environmental microbiome such as soil, water and air play an essential role todeepen biodiversity knowledge and compositional profiles in pollution context [1].To unveil microbiome role, it is essential to both unveil its taxonomic composition and assessits genetic potential reconstructing the component genomes as Metagenome-Assembled Genomes (MAG) [2]. To this aim, we developed kMetaShot, an accurate tool for MAGs taxonomic classification.

kMetaShot relies on kmer/minimizer counting and consists of two modules: the reference generator counts minimizers from RefSeq Bacterial and Archaea RNAs and stores relevant minimizers(i.e. exclusively shared among strains of the same genus) in a dedicated reference matrix; theclassification module processes MAGs, performs the minimizer counting and queries the referencematrix to achieve the taxonomic classification relying on a prevalence-based approach. kMetaShot has been benchmarked against two of the most cited comparable tools, i.e. GTDBtk[3] and CAMITAX [4] by classifying MAGsobtained by 20 rhizosphere in silico generated samples of the CAMI2 [5].

kMetaShot outperformed both GTDBtk and CAMITAX in term of precision at species (80%, 25%,30%, respectively) and genus (100%, 90%, 55%, respectively) levels. Differently from other tools, kMetaShot is able to perform classification at strain level with a Balanced-Accuracy at ~63%. kMetaShot is also the fastest and the most computationally effective.

kMetaShot is an easy-to-use, easy-to-install and computationally effective tool for MAGs taxonomic classification with high accuracy classification at strain level. It takes in input MAGs FASTA filesand is available and documented at https://github.com/gdefazio/kMetaShot and distributed as Conda package and Docker container.

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Presumptive identification of two Gram-positive microaerophilic strains isolated from Weddell's seals in Antarctica.

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The microbial biodiversity of bacteria inhabiting mucous membranes in the mouths of various animals in Antarctica is being investigated within the Czech Antarctic Research Programme (CARP).An analysis of palindromic PCR profiles of repetitive elements, carried out with the primer (GTG)5, was performed initially on a group of 100 microaerophilic Gram-positive bacterial isolates. The profiles of two strains isolated from the oral cavity of Weddell´s seals (*Leptonychotes weddellii*) during the Antarctic summer of 2021 showed fingerprints that differed significantly from all otherprofiles in the in-house database. Both strains, P13308 and P13329, were catalase-negative, microaerophilic, -hemolytic cocci belonging to Lancefield serological group C. The partial 16S rRNAgene sequence of the strains analyzed showed the highest similarity to the *Streptococcus pacificus* type strain CSL7591T. As the calculated similarity of the 16S rRNA sequences (98.5 %) was belowthe recommended threshold for species delimitation, there is a strong indication that the strainsstudied represent a novel species within the genus Streptococcus.

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New protocol development of bioinformatics analysis for species verification of the genus *Pseudomonas* from Polish Collection of Microorganisms resources.

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In the case of bacteria from the genus *Pseudomonas*, verification methods considered as the goldstandard, such as 16S rRNA gene sequence analysis and MALDI-TOF MS analysis, prove to be insufficient. The 16S rRNA sequence does not exhibit a significant degree of nucleotide polymorphism, making it difficult to accurately differentiate species within the genus. Additionally, MALDI-TOF MS analysis is inadequate due to the high similarity of protein profiles and the phenotypic variability of some species caused by environmental factors. Thus, more advanced methods are necessary to precisely identify bacteria from the genus *Pseudomonas*. Multi-Locus Sequence Analysis (MLSA) method relies on the analysis of multiple housekeeping genes, providing higherresolution of phylogenetic relationships of species within the genus.

The main aim of this study was to develop a protocol utilizing molecular biology methods and bioinformatics tools for the effective species identification of bacteria from the genus *Pseudomonas* deposited in the Polish Collection of Microorganisms (PCM).

Materials for the study included 11 bacterial strains from PCM: 4 reference strains and 7 strains for verification.

The methods used for initial identification included MALDI Biotyper and 16S rRNA sequencing. Further identification employed MLSA based on the housekeeping genes: gyrB, rpoD, rpoB, andrecA. Phylogenetic analysis was conducted using the Maximum Likelihood method.

The new protocol involving MLSA combined with phylogenetic analysis confirmed the identity of strains: PCM 1411 *P. syringae*, PCM 2856 *P. silesiensis* and PCM 2904 *P. laurysulfatovorans*. Strains PCM 1994 *P. fluorescens*, PCM 2124 *P. putida*, and PCM 2219 *Pseudomonas sp*. are proposed for reclassification to new species. PCM 2123 *P. fluorescens* was not clearly classified, so Whole Genome Sequencing is necessary in this case.

The obtained results proved that proposed protocol allows for more accurate identification of *Pseudomonas* species.

Novel insights into heterocyte patterning and proheterocyte germination in *Kaarinaea lacus* PCC 9237T gen. nov. sp. nov.

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Cyanobacteria are oxygenic photosynthetic prokaryotes and are broadly grouped into unicellularand filamentous forms. Certain filamentous cyanobacteria develop specialized cells called heterocyte that perform nitrogen fixation. These cells are terminally differentiated that not only differ functionally but also morphologically and physiologically from the other vegetative cells. Heterocyte differentiation is a complex and highly regulated process involving initiation, commitment, patterning, morphogenesis and maturation stages [1,2].
Among the heterocytous cyanobacteria, the genus *Nostoc* is one of the most widely studied genera.In this study, we conducted an in-depth characterization of a toxic *Nostoc sp*. 9237T isolated in 1986 from a freshwater bloom in Finland. Multilocus and 16S rRNA gene phylogenetic trees bothconfirmed that PCC 9237T represents a novel *Nostocalean* genus for which we propose the name *Kaarinaea lacus* gen. nov., sp. nov. Remarkably, PCC 9237T showed interesting morphological attributes such as unusual heterocyte patterning leading to the development of heterocytes in series followed by the fragmentation of trichomes at this site. This cyanobacterium displayed occasional proheterocyte germination, a morphological feature last reported more than five decades before [3,4]. We conducted a detailed genomic and morphological examination to gain a better understanding of these phenomenon. Furthermore, the genomic investigation for biosynthetic gene clusters (BGCs) revealed a significantly different heterocyte glycolipid (HG)BGC in PCC 9237T compared to the well-studied HG BGC of the model organism PCC 7120 [5]. In conclusion, the results obtained indicate *Kaarinaea lacus* PCC 9237T as a potential novel model organism to study proheterocyte germination and gain further insights into heterocyte patterning and differentiation.

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Exploring *Fusarium oxysporum* biodiversity in Beauvericin gene-cluster

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Fusarium oxysporum is classified as the fifth most important fungal plant pathogen in the world,is considered a species complex (FOSC), that includes over 106 formae speciales, known to infectmore than 100 different hosts where it causes vascular wilt. It can also produce a wide range of active metabolites, among which the mycotoxin beauvericin [1,2].
The biosynthetic gene cluster for beauvericin (Bea) consists of 4 genes: Bea1, encoding NRPS22, the non-ribosomal peptide synthase responsible for the synthesis of the beauvericin backbone, Bea2, Bea3 and Bea4, encoding proteins with transport and regulatory functions. A special role was discovered for the ABC transporter (Bea3): deletion of the coding gene led to a significant upregulation of Bea1 and Bea2 and to drastically increase product yields [3]. Understanding the genetic basis of beauvericin biosynthesis may provide insights into the regulation of biosynthesis, the potential for genetic manipulations and the development of strategies tocontrol the production of beauvericin in food and feed crops.
In this study, we analysed the biosynthetic gene cluster in *F. fujikuroi* and in 4 isolates of *F. oxysporum* f.sp. melonis that produce or do not produce beauvericin and originate from two differentareas: specifically, California in USA and Sicily in Italy. Bioinformatic comparison between *F. fujikuroi* and *F. oxysporum* in the BEA gene cluster revealed some differences in coding conserved regions, possibly non-affecting beauvericin biosynthesis. Specifically, in Bea3 an extra 56-bp region in exon 6 of *F. fujikuroi* is predicted as intron in *F. oxysporum*, generating an extra exon. Moreover, we found a difference in the Bea3 coding region, leading to a change in the amino acidsequence, apparently linked to different geographic origins of isolates.
Further studies are needed to understand whether this difference could be a marker to distinguish the origin of *F. oxysporum* isolates and to verify if it affects beauvericin production.

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Exploring cyanobacteria genomes for antithrombotic secondary metabolites

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Cyanobacteria are photosynthetic microorganisms capable of producing secondary metabolites with relevant bioactivities to human health, such as fungicidal activities, iron chelating, and protease inhibition. Anabaenopeptins, Microviridins, and Micropeptin K139 are some of the compounds produced with antithrombotic effect, being the anabaenopeptins capable of inhibiting Thrombin Activatable Fibrinolysis Inhibitor (TAFIa). Therefore, using resources from the Bank of Algae and Cyanobacteria of the Azores (BACA), the main goal of this work was to search for compounds with anticoagulant activities in 50 strains of cyanobacteria, using an OMIC approach. As a result,the genome of 50 cyanobacteria strains was obtained through Illumina sequencing. Genomic and phylogenomic analysis was performed and the search for biosynthesis gene clusters (gene mining) was conducted. To date, 22 of the strains under study, belonging to the genera *Azorothrix*, *Dulcicalothrix*, and *Nostoc*, among others, have shown genes capable of producing Anabaenopeptins, Microviridins, and Micropeptin K139, indicating potential for anticoagulant activities. The confirmation of the production of these compounds will be performed through metabolomic analysis, using HRLC-MS/MS and a bioassay to determine antithrombotic activity. Given that diseases such as thrombosis are one of the leading causes of death, these results may become promising forthe development of new anticoagulant drugs.

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GEN-ERA toolbox: reproducible bioinformatics workflows for genomics and taxonomy in culture collection.

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Microbial culture collections play a key role in taxonomy, as they facilitate the exploration of strain diversity and provide well-characterized biological material for both fundamental and applied research. To enhance species delineation, microbial resource centers are now adopting advanced methods such as whole-genome sequencing and phylogenomics. In line with this, the genomic needs of the Belgian Coordinated Collections of Microorganisms (BCCM) were studied, leading to the development of the GEN-ERA toolbox. This unified cluster of bioinformatic workflows is dedicated to both bacteria and small eukaryotes (e.g., yeasts). The GEN-ERA toolbox is publicly accessible and tailored for researchers lacking specialized bioinformatics training, operable with a single command line. Hence, it facilitates all steps from genome downloading and quality assessment, including genomic contamination evaluation, to phylogenetic tree reconstruction. Additionally, it supports workflows for average nucleotide identity comparisons and metabolic modeling. To enhance reproducibility, all workflows are containerized using Singularity and managed with Nextflow. The utility of the GEN-ERA toolbox is demonstrated through two studies focusing on bacteria and yeast. The first study establishes the presence of five species within the recently described cyanobacterial genus *Laspinema*, three of which are strains held in the BCCM/ULC collection. The second study involves phylogenetic analyses of the *Starmerella* genus, where we sequenced 21 genomes from the BCCM/MUCL collection, doubling the number of publicly available *Starmerella* genomes, including two high-quality long-read PacBio sequences. The results of the two studies highlight the importance of phylogenomics and GEN-ERA in interpreting evolutionary relationships and taxonomic delineation, particularly through the use of Average Nucleotide Identity.

Comparison study between molecular fingerprinting and FTIR BIOTYPER

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Within a microbial species there are different individuals carrying their own characteristics known as strains. Using the correct strain ensures consistency in biotechnological processes, leading to reliable and reproducible results. Identification of strains within a species can be done by molecular fingerprinting where it involves analysing the genome using different techniques such as RAPD and AFLP. Results can be obtained through gel electrophoresis or sequencing and creates a unique genetic fingerprint of the strain. Another technique is the Bruker's Fourier Transform InfraRed (FTIR) spectroscopy system, the IR Biotyper. FTIR spectroscopy measures the absorption of infrared light by chemical bonds and the resulting spectrum represents the molecular composition which can be used to distinguish strains based on their biochemical signatures. It is reliable and fast; however, IR Biotyper protocols were developed for bacteria. Because the reading of the plate is done with the incidence of infrared light it is essential to have a homogeneous solution and since filamentous fungi have complex, filamentous structures, ensuring a representative and homogeneous sample can be difficult.
In this sense, a filamentous fungi protocol to be used in the IR Biotyper was developed and compared with the results obtained in the molecular fingerprinting. For this, 16 strains of *Penicillium crustosum* with different origins (country and commodity) were used. Molecular fingerprinting was obtained by $(GACA)_4$ and M_{13} primers amplification and the recommended protocol for bacteria was modified. Molecular fingerprinting showed that some strains created clusters according to their origins. The preliminary tests showed that the modified protocol is suitable for this species and the results of the IR Biotyper seem to be consistent with the fingerprinting results. However, additional strains are going to be analysed as proof of concept.

Acknowledgements
This work was supported by the Portuguese Foundation for Science and Technology (FCT) under the scope of the strategic funding of UIDB/04469/2020 unit and LABBELS – Associate Laboratory in Biotechnology, Bioengineering and Microelectromechanical Systems (LA/P/0029/2020). "MIRRI-PT (Polo Norte)" project (PINFRA04/84445/2020) funded by the European Regional Development Fund under Norte2020-Programa Operacional Regional do Norte allows the establishment of the molecular biology and proteomic platforms. Teresa Vale Dias acknowledges FCT for the PhD grant 2020.05849.BD.

Advancing research in synthetic biology through DTU Bioengineering's Fermentation and HT-automation facility

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Synthetic biology has emerged as a revolutionary field with vast potential to address global challenges ranging from healthcare to environmental sustainability. Central to this endeavor is the development of efficient fermentation platforms capable of producing a variety of bio-based products. We herein present insights into the advancements achieved through a state-of-the-art fermentation platform facility dedicated to synthetic biology research and upstream bioprocess design.

The facility serves as a nexus for interdisciplinary collaboration, bringing together experts from biology, chemistry, engineering, and computational sciences. Through meticulous design and optimization, the facility enables custom design and optimization over a wide array of microbial fermentation processes, facilitating the production of complex biomolecules with tailored functionalities.

Key highlights include the utilization of novel genetic constructs and metabolic engineering strategies to enhance product yields and diversify output. Moreover, the integration of high-throughput screening techniques expedites the identification and optimization of microbial strains for specific applications.

Case studies illustrate our facility's versatility, ranging from the sustainable production of microbial based biofuels and pharmaceuticals to the synthesis of biodegradable polymers and nutraceuticals. Overall, this work underscores the pivotal role of the DTU fermentation core facility in advancing synthetic biology, paving the way for developing innovative solutions to address societal and environmental challenges.

Identification and characterization by a polyphasic approach of the microbial agent responsible of spoilage in Grana Padanocheese

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A peculiar alteration of microbial origin in Grana Padano (PDO) cheese was investigated. The defect appeared after 6–7 months of ripening as whitish spots located in the innermost parts of the cheese wheel and evolved with a degradation of the cheese matrix, followed by a formation of spongy cavities. Portions from both spoiled (S) and non-spoiled (NS) cheese areas were sampled and analyzed. Butyric clostridia spores (MPN g-1) accounted 2.5 log in S areas compared to <-0.5 in NS ones. Metataxonomic analysis showed *Clostridium spp*. as the most abundant taxon in all the samples, with two distinct *Clostridium spp*. taxa dominating in NS and S areas, respectively. From the positive MPN tubes of both S and NS samples, 24 strains were isolated, identified as *Clostridium (C.) sporogenes* by species-specific PCR, and grouped into four distinct genotypes after RAPD-PCR fingerprinting analysis. One of the four genotypes included only isolates from NS-areas whereas the other three included isolates from the S-areas. 16S RNA sequencing of the dominant genotype of the S areas showed a 5 bp sequence polymorphism within the first 500 bp compared to the sole genotype of the NS areas. High-Resolution Melting applied to two strains representing these two genotypes showed two distinct profiles, which further suggests a taxonomic heterogeneity between them. Taken together, results confirmed the involvement of *C. sporogenes* in the onset of the defect of the cheeses studied. Genotypic and phenotypic characterization suggested the presence of two different prevailing biotypes of *C. sporogenes*, corresponding to two separate genetic lines, with notable morphological and taxonomic differences, isolated respectively from spoiled and defect-free zones of the cheeses. Further chemotaxonomic analysis is underway to clarify the taxonomic affiliation of the two distinct *Cl. sporogenes* genotypes isolated in this study.

The importance of the *Legionella surveillance* approach implemented in the company's water distribution system to discover a novel *Legionella* species: *Legionella petroniana* sp. nov.

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The study presents the description and characterization of a novel *Legionella* species isolated in Italy during a *Legionella* surveillance program developed in a company, where a risk assessment plan has been implemented since 2015. Sampling was conducted on cold and hot water distribution systems (WDS), following ISO19458:2006, and *Legionella* isolation was performed according to ISO11731:2017. *Legionella* isolates were identified using serological test and MALDI-TOF MS technique. Moreover, *mip* and *rpoB* gene sequencing, other than whole genome sequencing (WGS), were performed for *Legionella* species identification. In 2018, a *Legionella* contamination at a concentration of 400 +CFU/L was detected in a single cold point of WDS, located on the terrace of the building, used for air handling unit (AHU) cleaning and maintenance procedures. The colonies showed differences in terms of morphology with respect to the common *Legionella* features, although growth was only on BCYE with L-cysteine(Cys+). The isolates characterization process provided the following results: cells were Gramand Ziehl Neelsen-stain-negative, rod-shaped, and motile. The strains grew in a range of 32–37 °C on Cys+, GVPC, and MWY agar medium, with a positive reaction for oxidase and gelatinase. The dominant fatty acids were summed features 3 (C16:17c/C16:16c) (28.9%), C16:0 iso (18.4%), and C15:0 anteiso (15.4%), and Q13 as the major ubiquinone. The *mip* and *rpoB* gene sequences showed a similarity of 98.2% and 95.1%, with *L. feeleii* (ATCC 35072T). The GC content was 41.37 mol%, and the digital DNA-DNA hybridization (dDDH) analysis returned a value of 54.5% DNA-DNA relatedness with *L. feeleii* (NCTC 11978T). The Average Nucleotide Identity (ANI) between the strains and *L. feeleii* (NCTC 11978 T) was 94.02%, confirming that the isolates represent a novel species of the genus *Legionella*. The name proposed for this species is *Legionella petroniana* sp. nov., with 31fI33T (=DSM 114357T=CCUG 76442T) as the type strain.

Session 8 – Networking, services, quality and data management of Microbial culture collections

Friday 20th September, 11.45:00am

Chairs: Lima Nelson (Portugal), Verkley Gerard (Netherlands).

MIRRI-ERIC as a microbial, genetics and data resources hub to advance scientific discovery

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The Microbial Resource Research Infrastructure (MIRRI-ERIC), recognized as an ESFRI Landmark, serves as a linchpin for advancing bioscience and bioindustry endeavors. It offers a centralized platform for accessing diverse, high-quality bioresources and associated data, fully compliant with European-level data policies. Supported by a global network of microbial research centers and experts, MIRRI-ERIC fosters collaboration aimed at safeguarding microbial biodiversity, driving scientific inquiry, and nurturing professional development within the field. Presently, MIRRI-ERIC has six members (Belgium, France, Greece, Latvia, Portugal, and Spain) and one observer (Romania). Several other countries have shown interest in joining MIRRI-ERIC and contributing to strengthening its role as a hub for microbial, genetic, and data resources to advance scientific discovery. MIRRI-ERIC coordinates the Microbes4Climate Horizon Europe (HE) project, with the participation of two other research infrastructures (RIs), AnaEE-ERIC and LifeWatch-ERIC, along with twenty-eight research and innovation organizations. This initiative aims to provide researchers worldwide, including those focused on biodiversity, with streamlined access to a suite of integrated services, in harmony with the European Green Deal and the Digital Single Market Strategy. Additionally, MIRRI-ERIC, together with EMBRC-ERIC, ELIXIR, INSTRUCT-ERIC, four companies, and sixteen research and innovation organizations, was recently awarded the MALDIBANK project. This project aims to build a global cloud-based multi-domain open MALDI spectra bank for the identification of microorganisms. MIRRI-ERIC is reshaping its services and is deeply committed to making its culture collections FAIR—findable, accessible, interoperable, and reusable. These efforts align with the European Union's Digital Agenda and the European Strategy for Data, fostering innovation and knowledge exchange while safeguarding biodiversity for future generations.

Acknowledgements
The author is greatly indebted to MIRRI-ERIC and MIRRI-Nodes People, and to Microbes4Climate and MALDIBANK teams. Microbes-4-Climate – Project No. 101131818 is funded by the European Union's Horizon Europe program. MALDIBANK will be funded by the European Union's Horizon Europe program.

How the MIRRI-PT has been developed in its smart specialisation and cutting-edge technologies to offer better services

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MIRRI-PT brings together 11 microbial culture collections from different regions. MIRRI-PT is well aligned with the domains of the "Regional Smart Specialisation Strategies", namely: i) "Agroenvironmental Systems and Food"; ii) "Life Sciences and Health; iii) "Sea Resources and Economy" and iv) "Human Capital and Specialized Services". In domain (1) we find the most relevant goods produced and exported include fermented alcoholic beverages, dairy products, and other agricultural products. MIRRI-PT has among its members that having a proven track record of collaboration with startups, SMEs, large companies, and regional farmer organisations in the referred sectors. In domain (2) we find a considerable amount of consolidated companies in medical devices, which have been mostly active in segments with a not very high incorporation of knowledge/technology, along with the youngest, smaller companies that target high-tech, high added-value segments. MIRRI-PT intends to strengthen its research capabilities and broaden the reach of services it provides. In the domain (3) MIRRI-PT is engaged in biotechnological activities associated with these microbial resources that will be the source of new products with applications in industry: antifouling compounds, biomaterials with application in food and health, and colourants for food and textile industries. Microbial resources may also be important for the development of microalgae and cyanobacteria-based biodiesels and biofuel as well as for the development of feed used in aquaculture. In domain (4) the approach has been the development and launch of an online platform featuring an e-catalogue of microbial resources, associated data and specialised services, to boost and facilitate access to the different user communities. This is an example of the efforts to promote digital transformation and the dematerialisation of processes. Finally, acquiring new equipment allows MIRRI-PT to offer new cutting-edge technologies and services.

Acknowledgements
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PLAVIT, the Italian Plant Virus Collection in 2024

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Viruses still generate major concern in public opinion, because mainly considered as human pathogens. However, they are more and more recognized as essential within ecosystems to maintain an ecological balance among different microorganisms and living beings in general. Therefore, viruses are key to environmental and human health, to science as well as to bioeconomy and biotechnology applications. Hence the need to develop and improve collections and biobanks not only of human viruses, but also of plant, microbial, and environmental viruses.

Plant virology started in Italy in the late 60's when specific expertise and equipment became available and new diseases of unknown etiology were studied. At that time virus identification was limited to bioassays on test plants, electron microscopy and basic serological tests. Dried leaf samples were collected and stored, mainly in laboratories of the National Research Council of Italy (CNR) located in Torino and Bari. The Plant Virus Italy (PLAVIT) collection, which includes more than 1,000 isolates, began to be redeveloped and quality-checked starting from 2020 thanks to dedicated European and national funds. The process, consisting of several steps (revitalization, authentication, sequencing, preservation, storage, distribution) is implemented in the frame of a Quality Management System and of international guidelines specifically developed for viruses that define mandatory information necessary for an isolate to be included into the PLAVIT catalogue. Up to now approximately half of the virus and viroid isolates present in the collection underwent the quality check and can be distributed upon request to academic and private research centers. In the framework of a continuous improvement scenario, more isolates will be processed, new ones will be acquired, and the categories in the catalogue will be expanded to include mycoviruses, bacteriophages and phytoplasmas, as well as nucleic acids and detection tools.

Acknowledgements
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The italian network of microbial culture collections: an overview on management and sustainability of the future research infrastructure MIRRI-IT

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Majority of Italian microbial Culture Collections (mCCs) belong to the Joint Research Unit (JRU) MIRRI-IT, a network aiming at improving quality management system and addressing needs of stakeholders operating in the biotechnology transfer. Several institutions are currently undergoing an intensive “restyling” due to the NextGenerationEU-funded project SUS-MIRRI.IT, which focuses on the implementation of mCC management and offer of services. The creation of the National Node of a Research Infrastructure (RI) that will coordinate the network of Italian mCCs, under the umbrella of MIRRI-ERIC, the Microbial Resource Research Infrastructure-ERIC, is recognized as valuable upgrade to get recognition and accountability at international level, to garner support from stakeholders, to attract funding, and to foster continued and fruitful collaborations. However, beside these advantages, memberships in a RI requires compliance with several obligations and adoption of rules. The big challenges to overcome are the implementation of the quality management system according to International Standards; the compliance with legal frameworks; the digital information and technology access guided by the FAIR (Findable, Accessible, Interoperable, and Reusable) principle; the continuous amended capacity building to stay at the forefront of science and maintain stakeholder expectations, the top-level training of the personnel, the collaboration with policy-makers and industry; and, last but not least, the guaranteeing of a future financial sustainability. The situation of the Italian mCCs will be presented proposing a possible governance of the Italian RI MIRRI-IT after the end of the SUS-MIRRI.IT project. Moreover, a focus on the environmental, scientific, and socio-economic impact of this RI will be highlighted presenting stakeholder engagement, value proposition, collaborations with private sector, partnerships with national and local authorities, and funding strategy.

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Acknowledgements

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Managing change within a culture collection: A case study on the challenges within quality, data management and infrastructure within CABI's collection

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Established in 1947, the CABI Genetic Resource Collection (GRC) is one of the most significant global collections of microorganisms, containing >28,000 fungi and approximately 2,000 bacteria. The collection is maintained through a combination of core funding from 48 member counties, commercial services and research projects. Until recently, commercial services were the focus, with some microbial services operating with ISO17025:2017 accreditation. A recent science-focused CABI review has resulted in the GRC's positive move to better supporting CABI's mission, member countries and project research activities, with an associated managed reduction in third-party audited commercial services. Quality is being maintained by refocussing resources on our other regulatory responsibilities such as plant health licensing, biological safety and the Nagoya protocol/CBD, contributing to recent works by the FAO on DSI regulation. Additional resources have been allocated to biological maintenance activities associated with supply of cultures and data management related to managing workflows within the collection. We have also been exploring ISO20387:2018 as a more relevant quality standard and to this end we maintain a presence on the steering group for the Biobank standard in the United Kingdom Accreditation Service (UKAS). Any of our remaining commercial activities either have a direct benefit to GRC operations or are performed with a consultation style approach that fits better with our mission and our expanding research portfolio. CABI's UK science centre is relocating to Imperial College's Silwood Park campus in 2025 with associated infrastructure upgrades for the GRC. In preparation, a programme of digitising all historic records relating to our strains has begun. Whilst the diversity of the archives provided challenges, it has positively impacted our ability to examine the provenance of strains when performing assessments on Nagoya compliance.

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Establishment of the ITEM Culture Collection backup and development of the related disaster recovery plan

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The development of microbial and microbiome-based solutions is decisive in delivering progress and sustainable innovations in agro-food systems. In this contest, Culture Collections (CCs) have a key role in ensuring scientific progress, promoting bioeconomy. The ITEM Microbial Culture Collection of ISPA (Institute of Sciences of Food Production, CNR, Bari) includes more than 13,000 strains belonging to various agri-food microorganisms (filamentous fungi, yeasts, and bacteria). In the framework of the European-funded SUS-MIRRI.IT project, which aims to improve the management and sustainability of Italian microbial collections, ITEM collection is implementing a disaster recovery plan to prevent accidental loss that includes an additional storage as "safe backup" in Foggia, about 130 kilometers from the primary territorial unit (ISPA-CNR, Bari). This safe storage facility includes a dedicated room with new devices for cryopreservation at -150°C and -80°C. As part of the project activities, a new molecular biology laboratory has been set up, including a capillary sequencer of nucleic acids, a spectrophotometer for nucleic acid and protein measurements, and an imaging system for molecular biology. A laboratory for preparatory procedures completes this infrastructural effort. All these facilities are also functional to restore the correct operation of ITEM collection in the occurrence of an emergency or unexpected negative event in the headquarters. Strategies are being implemented for the preservation of digital information (microbiological passport data). Tailored training activities are ongoing to ensure the appropriate recovery, transfer and cryopreservation of the microorganisms in the safe storage facility. Furthermore, we highlight how the different research lines active in the secondary territorial unit of Foggia will represent the driver of innovation to increment and exploit the micro-biodiversity preserved in the ITEM culture collection.

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A cloud-based, open-access platform for comprehensive metagenomic data analysis

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The Italian Node of the Microbial Resource Research Infrastructure, overseen by the Joint Research Unit MIRRI-IT, is essential for advancing microbial research in Italy. In 2022, the Italian government allocated €17 million from the NextGeneration EU-funded PNRR to support the SUS-MIRRI.IT project. Since microbial resources include extensive metadata and encompass high-throughput sequencing datasets, within the SUS-MIRRI project, we have worked to create metagenomic analysis workflows and to implement them as an online service orchestrating various software within an HPC architecture. To ensure accessibility, we implemented a web user interface using Next.js. The workflow implementation utilises StreamFlow [1], a Workflow Management System based on the Common Workflow Language standard, to be executed within the HPC system using SLURM [2], to allow multiple workflows to run simultaneously.

To show the effectiveness of our approach, we present here two workflows. The first one is designed for microbial genome assembly: it supports long-read assembly by harnessing various assembler tools [3,4,5], then polishes and merges the results to improve the outcome. The second one performs metagenomic analysis, including taxonomic annotation and microbial population characterisation through: i) reading alignment for host removal and taxonomic classification using Kraken2 [6] and MetaPhlAn4 [7], ii) post-processing for normalisation, decontamination, filtering, abundance estimation, and diversity calculation, and iii) metadata analysis and regression model computation. These two workflows demonstrate our service's scalability by varying the number of computational resources.

Our platform empowers scientists with remarkable computing power, unavailable to most laboratories, and supports their research endeavours with an interactive and clever environment. By leveraging interdisciplinary knowledge, our platform enables the exploration of results from multiple perspectives.

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Strengthening the CoSMi culture collection: a contribution to preserving the biodiversity of microalgae

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Microalgae are unicellular autotrophic organisms that produce half of the oxygen we breathe andremove carbon dioxide from the atmosphere through photosynthesis. They are the main primaryproducers of biomass for aquatic systems, and thus support life on earth. In addition to thiskey ecological role, their potential importance for biotechnology has emerged in last decades. Microalgae are indeed a diverse group, containing a large amount of proteins, lipids, carbohydrates, pigments, -3 fatty acids and other chemicals that could serve as feedstock for various products indifferent fields such as pharmaceuticals, cosmetics, food and feed, but also as an alternative source of renewable fuels. Against this background, culture collections play a key role in protecting and preserving this invaluable microalgae biodiversity. However, these collections are often the result of the research needs and efforts of individual researchers, so they do not meet the relevant international standards.
The Collection of Sea Microorganisms (CoSMi) at the National Institute of Oceanography and Applied Geophysics (OGS) is one of the few official collections specialised in marine microalgae. It is part of the WFCC and is linked to national and international research infrastructures such as MIRRI.IT, LifeWatch, ECCSEL and EMBRC ERIC, as well as being an integral part of the BioMarine Lab and the Gulf of Trieste Observatory System. In the framework of the Italian PNRR project SUS-MIRRI.IT, promoted by JRU MIRRI.IT, the CoSMi collection will be strengthened by: 1) improving technological equipment and scientific instruments; 2) better characterization of microbial biodiversity through morphological and molecular analyses; 3) developing and implementing standard procedures for sampling, characterization and conservation; 4) improving online accessibility of information by implementing and publishing the local database and harmonising it with the national database and by upgrading the website.

The SUS-MIRRI.IT project: research, services, and training for sustainable bioscience and bioeconomy by the MIRRI Italian Research Infrastructure

Arena, Mattia Pia[1] ON BEHALF OF THE SUS-MIRRI.IT PARTNERS

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SUS-MIRRI.IT is a project funded by the NextGenerationEU programme, with a total budget of about € 17,000,000. The project involves 24 Italian Operative Units.
The mission of SUS-MIRRI.IT is to strengthen the Italian network of Culture Collections (CCs) holding different microbial resources, including bacteria, filamentous fungi, yeasts, microalgae and viruses. The allocation of funds is aimed at the acquisition of cutting-edge equipment, infrastructural improvements, recruitment of high-qualified staff, strengthening of the preserved microbial resources with associated data, and top-level services. The project is organized into 6 Work Packages (WPs): WP1 drives management, governance, and sustainability; WP2 is involved in microbial CCs consolidation; WP3 focuses on digital activities for the creation of the single-entry point to the National Database of microbial resources and services; WP4 aims to study the biotechnological valorisation of microbiomes; WP5 deals with the implementation of offered services and training courses; WP6 is dedicated to communication and dissemination actions.
To date, over 40 researchers, PhD students, and technicians have been hired; about 70% of the funds for innovative technologies have been utilized; the online platform including the catalogue of Italian microbial resources has been launched; 19 advanced training courses have been delivered, with a participation rate of over 900 attendees; 30 scientific papers have been published. Allproject updates are communicated by the dedicated website and related social media.
The strategic impact of the project is to promote partnerships on the territory and encouraging theaggregation of skills, structures, and bioresources. Targeted initiatives and stakeholder-oriented actions will ensure that results are disseminated and, when possible, transferred to industry, promoting the transition towards the green economy, and contributing to the sustainability and safetyof the environment.

The Usage and Fate of Patent Deposits

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Since 1981, the DSMZ has been recognised as an International Depositary Authority (IDA) underthe Budapest Treaty. The tasks of an IDA are clearly defined: Deposit of samples for patent purposes and provision of biological material to authorised parties. The DSMZ holds more than 9,000 patent deposits, that are mentioned in more than 24,000 patents (Scopus, using "DSMZ" as a search parameter). However, many patent deposits never make it into a patent, either because the deposited material does no longer fall within the scope of the depositor or the Budapest Treaty deposit is "misused" for other purposes. Many patent deposits are also mentioned in scientific publications, although this form of deposit may not be in line with the need for public availability, especially when type strains are involved. Interestingly, DSMZ patent deposits also appear in EFSA publications. This is presumably because EFSA requires that “organisms to be evaluated should be deposited in an internationally recognised culture collection that has acquired the status of an international depository under the Budapest Treaty" [1], which refers tothe organisation of the culture collection but not the form of deposit. In this case, a safe deposit would be recommended. Considering that patent deposits can be used for other purposes than intended, we would like to point out that it is not clear what should happen to these deposits after the mandatory storage period of at least 30 years after the date of deposit (Rule 9.1 Budapest Treaty), with options ranging from destruction to open availability of the material. We would therefore like to continue the discussion on what should happen to these deposits after thedescribed storage period, considering various aspects such as the disclosure of patents, the open availability of the material and the feasibility for IDA collections. As well as asking whether otherdefined forms of deposit are needed to fulfil stakeholder requirements.

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Performing an upgraded and interoperable data management system for ITEM Microbial Culture Collection

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ITEM is an internationally recognized and ISO 9001-certified culture collection, hosted at the CNR Institute of Sciences of Food Production (ISPA) in Bari (Italy). ITEM holds more than 14.000 strains of agro-food interest including filamentous fungi, yeasts and bacteria. It is a partner of the Italian network of microbial resources collections "JRU MIRRI-IT" (https://mirri-it.it/) and hasbeen involved in the MIRRI-ERIC Infrastructure constitution (https://mirri.org). In addition to the ITEM database, strains-related data are included in the i) World Data Centre for Microorganisms (https://wdcm.org/), ii) MIRRI-ERIC Catalogue and iii) CNR Biomemory Project network (https://biomemory.cnr.it/).
Thanks to the support of the ongoing project "SUS-MIRRI.IT", ITEM is upgrading and improvingboth the collection database and infrastructure, to integrate all the information associated with the strains preserved, improve quality control management and setting up a disaster recovery planby creating a physical backup at the CNR-ISPA institute in Foggia (Italy). Generally, collections databases should not be standalone systems, but they must interact with other bioinformatics systems to achieve a global view of data and passport information available on strains [1]. Accordingly, the ITEM upgraded database management relies on interconnections among different platforms storing biological data and microbial culture data: BioloMICS (BioAware) for on-line catalogue, base statistics and images DB (https://item.bio-aware.com/); EasyTrack2D (Twin Helix) for internal physical storage reference; Bionumerics (Applied Maths) for DNA/RNA sequences and passport data. All information is locally managed by R scripts (R Core Team) to conform different standards.
The final goal is to standardize all information under FAIR principles (https://go-fair.org/), in a unique platform with Application Programming Interface capabilities to intercommunicate with other external components.

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"Strengthening the MIRRI Italian Research Infrastructure for Sustainable Bioscience and Bioeconomy" research project (acronym: SUS-MIRRI.IT; European Commission – NextGenerationEU code n. IR0000005

Bank of Algae and Cyanobacteria of the Azores – BACA culture collection

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The Bank of Algae and Cyanobacteria of the Azores (BACA) culture collection was created in 2018reuniting several microalgae and cyanobacteria culture collections from the University of the Azores (Portugal) in one unique collection, guaranteeing quality and long-term maintenance of these important genetic resources. Since then, BACA has reached 800 cultured strains of cyanobacteria and microalgae. Is a member of ECCO and Pt-mBRCN since 2021, promoting access to its cultures through a public catalog (baca.uac.pt). The BACA culture collection is a hub of biodiversityallowing for several studies on taxonomy, with several novel genera and species published, supporting the conservation of these unique genetic resources isolated from the Azores archipelago. Several works have been published regarding the toxicity of cyanobacteria but also taking into account biotechnological applications, valorizing the BACA culture collection as a source of important compounds. Recently, more than 100 genomes of cyanobacteria from the BACA culture collection have been sequenced by Illumina, creating high-quality draft genomes, which further support genetic and biotechnological studies.

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By Portuguese National Funds, through FCT—Fundação para a Ciência e a Tecnologia, the European Union, QREN, FEDER, COMPETE, by funding the CIBIO/InBIO (project UID/BIA/ 50027/2013 and POCI-01-0145-FEDER-006821).

A ranking tool for microbial biobanks

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Microbial biobanks preserve and provide microbial bioresources for research, training, and quality control purposes. They ensure the conservation of biodiversity, contribute to taxonomical research, and support scientific advancements. Microbial biobanks can cover a wide range of phylogenetic and metabolic diversity, or focus on specific taxonomic, thematic, or disease areas. The strategic decisions about strain selection for certain applications or for the biobank culling necessitate a method to support prioritization and selection.
Here, we propose an unbiased scoring approach based on objective parameters to assess, categorize, and assign priorities among samples in stock in a microbial biobank. We describe the concept of this ranking tool and its application to identify high priority strains for whole genome sequencing with two main goals: (i) genomic characterization of quality control, reference, and type strains;
(ii) genome mining for the discovery of natural products, bioactive and antimicrobial molecules, with focus on human diseases. The general concept of the tool can be useful to any biobank and for any ranking or culling needs.

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A shared quality management system for Italian Culture Collections: The challenge of SUS-MIRRI.IT project

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Quality management system (QMS) implementation is one of the main goals of the European Microbial Resource Research Infrastructure (MIRRI-ERIC), which operates actively on the design of standard guidelines. Culture collections (CCs) must manage the quality of their bioresources and guarantee the deposited microbial strains' viability, purity, and identity according to internal standard operation procedures (SOPs). In this respect, the SUS-MIRRI.IT project aims to harmonize and improve the QMS of the Italian network of CCs. Within the activities of the project, three working groups dealing with (i) Common Collections Procedures, (ii) Compliance with the Nagoya protocol, and (iii) Code of Conduct on Biosafety and Biosecurity, are working to define a general and harmonized management system for the Italian CCs. The description of the commonprocedures is still in progress with the definition of SOPs for accepting microbial cultures, their quality check, and protocols for microorganisms' preservation and distribution. Regarding the Legal compliance with the Nagoya protocol, starting from the Material Transfer Agreement (MTA) and Material Deposit Agreement (MDA) models recently published [1], a common document implementing MTA and MDA for the Italian CCs has been developed and proposed with the supportof an external expert in law. The QMS should also address the capability of collections to meet allrelevant national and international rules concerning the safe distribution of different bioresources. Accordingly, the working group on biosafety and biosecurity, which includes experts on human, animal, and plant diseases, is implementing guidelines for handling microorganisms based on their risks. The adoption of common rules for quality management is a challenging goal of primary importance for the CCs involved in SUS-MIRRI.IT to build a valid national network capable of responding to the needs of public and private actors in the fields of research and bioeconomy.

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Acknowledgments
"Strengthening the MIRRI Italian Research Infrastructure for Sustainable Bioscience and Bioeconomy" research project (acronym: SUSMIRRI.IT; European Commission – NextGenerationEU code n. IR0000005)

The ENEA Microbial Culture Collection as valuable resource for developing new sustainable biotechnologies and bioeconomy

Bevivino, Annamaria[1]; Ambrico, Alfredo[1]; Molino, Antonio[1]; De Rosa, Luigi[1]; Colantoni, Eleonora[1]; Casella, Patrizia[1]; Tabacchioni, Silvia[1]; Alisi, Chiara[1]; Costanzo, Manuela[1]; Lico, Chiara[1]; Bianco, Linda[2]; Cuna, Daniela[1] and the ENEA's team of SUS-MIRRI.IT project

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The ENEA microbial culture collection (EMCC) has been created over the course of 30 yearsby an interdepartmental team of researchers that have collected microorganisms and microbial consortia from different environments, like contaminated sites, hypogea and archaeological sites, food, lake sediments, sea, soil, rhizosphere, water [1]. The microbial collection has several importantapplications ranging from the health and quality of soil and cultivated plants to the degradation of environmental contaminants, from the production of bio-based molecules for industrial, energy and food uses to new products for the restoration of artistic heritage, from the development of functionalized packaging to the support of food quality and safety [2]. EMCC's mission is to isolate, identify, characterise, collect, store and distribute the microbial resources and related information, to improve their characterization and optimise their management, thus unlocking their genomic and metabolic potential. EMCC collection actually spans over 1400 microbial strains including mainlybacteria, but also fungi, yeasts, two microalgae and a plant virus. The most abundant genera are *Burkholderia*, *Pseudomonas*, *Bacillus*, *Arthrobacter*, *Streptomyces*, *Paenibacillus*, *Staphylococcus*, *Stenotrophomonas*, and *Microbacterium* for bacteria, *Aspergillus*, *Penicillium*, *Engyodontium*, *Cladosporium*, and *Trichoderma* for fungi, and *Saccharomyces*, *Rhodotorula* for yeasts. All the information i.e, the taxonomic assignment, origin, geographical geolocation, growth conditions and fields of application, is stored in a relational database, accessible through a web application and easily consultable using search keys that allow you to get the desired data. The preparation of bacterial starter in 2-5 L bioreactors and the scaling-up of the process in 15, 50 and 500-liter represent one of the important services that the EMCC will provide over the years to third partiesin the agricultural and novel food sectors.

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Speaking the same language: Changing the way to share microbial strain data

Whitte, Julius Fredrich[1]; Reimer, Lorenz Christian[1]; Yurkov, Andrey[1]; Bunk, Boyke[1]; López-Coronado, José Miguel[2]; Alokozay, Susan[2]; Zuzuarregui, Aurora[2]; Aznar, Rosa[2]; de Vries, Ronald P.[3]; Robert, Vincent[2]; Overmann, Jörg[1]

[1] Leibniz Institute DSMZ -German Collection of Microorganisms and Cell Cultures GmbH, Braunschweig, Germany; [2] Spanish Type Culture Collection-University of Valencia (CECT-UVEG), Paterna, Spain; [3] Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands

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Microbial strain data is often highly unstructured or the structure is tailored to only fit one system.To solve this problem we looked at the existing and historical standards for strain data and cameup with an modular, flexible and technical up to date solution, integrating existing schemas. In addition to this linked data we will also develop a technical infrastructure providing online tools to support and use this format.

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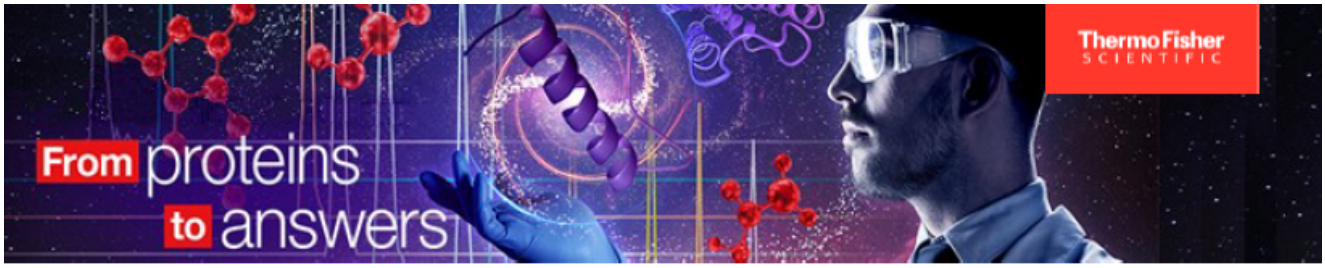

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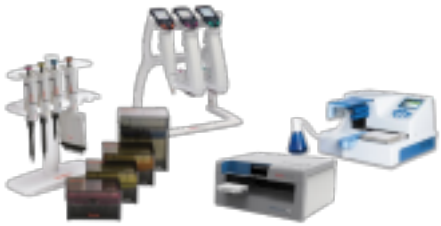

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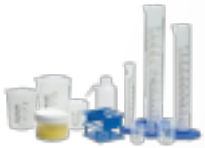

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