UNIVERSITÀ DI PARMA

SIMTREA

7TH INTERNATIONAL CONFERENCE ON MICROBIAL DIVERSITY

AGRIFOOD MICROBIOTA AS A TOOL FOR A SUSTAINABLE FUTURE

September 26-29 • 2023
PARMA, ITALY

Book of Abstract

MICROBIAL DIVERSITY 2023

AGRIFOOD MICROBIOTA AS A TOOL FOR A SUSTAINABLE FUTURE

UNIVERSITÀ DI PARMA

SIMTREA

SESSIONS

01 FOOD microbiota as a tool for a sustainable future

02 HUMAN microbiota as a tool for a sustainable future

03 ENVIRONMENT microbiota as a tool for a sustainable future

04 Exploiting microbiomes for a sustainable future

05 A sustainable future has come

TUESDAY 26 | September 2023

PALAZZO CENTRALE UNIVERSITÀ DI PARMA
Via Università 12, 43121 Parma

16:00 Registration

16:45 Institutional greetings

17:00 **Opening lecture: "How to manage with fermentation microbiomes: metabolic framework of spontaneous versus synthetic metacommunities"**
Marco Gobbetti, (Free University of Bolzano, Italy)
Chair: Rosalba Lanciotti, Erasmo Neviani

18:00

WELCOME CEREMONY

Atrio delle Colonne,
Palazzo centrale Università
di Parma
Via Università 12, 43121 Parma

WEDNESDAY 27 | September 2023

PAGANINI CONGRESSI
Via Toscana 5/a, 43121 Parma

08:30	Registration

SESSION 01 | FOOD microbiota as a tool for a sustainable future

Chair: Paul Cotter, Eugenio Parente

09:00	**Plenary lecture: "A MASTER Plan: Leveraging food and food chain microbiome data for a sustainable future" Paul Cotter, (Teagasc & SeqBiome, University of Cork, Ireland)**
10:00	Twenty years of investigation on riboflavin overproducing food-grade bacteria: Biotechnological applications and perspectives, **Pasquale Russo**, (University of Milano, Italy)
10:20	Back to the complexity: the challenge of natural starter cultures development, **Luigi Chessa**, (Agris Sardegna, Italy)
10:40	Dairy environment and seasons affect the microbiome of artisanal cheese, **Ilario Ferrocino**, (University of Torino, Italy)
11:00	**COFFE BREAK**
11:15	Poster session
11:45	Dynamics and acidification properties of raw milk bacterial communities during serial fermentations, **Chloe Gapp**, (University of Lorraine, France)
11:55	Variation of microbiota, chemical composition and B-vitamins in milk from alpine pasture and indoor dairy cows, **Giorgia Secchi**, (Edmund Mach Fundation, Italy)
12:05	Microbiome of kefir produced using Amiata donkey milk: characterization of the microbial communities and of fatty acid profile, **Matteo Daghio**, (University of Firenze, Italy)
12:15	Characterization of lactic acid bacteria in pursue of potential candidates for starter, adjunct and probiotic cultures, **Maria Aspri**, (Cyprus University of Technology, Lemesos, Cyprus)
12:30	**LUNCH**

SESSION 02 | HUMAN microbiota as a tool for a sustainable future

Sarah Lebeer, Marco Gobbetti

14:00	**Keynote: "The uniqueness of the microbiome of the human reproductive tract" Sarah Lebeer (University of Antwerp, Belgium)**
14:30	Environmental pollution drives adaptation in gut microbiome functions of highly exposed individuals, **Francesca De Filippis**, (University Federico II, Napoli, Italy)
14:50	Development of inhalation powders containing lactic acid bacteria: a way to the lungs to possibly boost respiratory health, **Benedetta Bottari**, (University of Parma, Italy)
15:10	Sourdough fermentation supports in vitro eubiotic effects of gluten-free bread fortified by insect flour, **Andrea Gianotti**, (University of Bologna, Italy)
15:30	**COFFEE BREAK**
15:45	Poster session

16:15	The middle-term intake of hydrolyzed and fermented arabinoxylan-oligosaccharides (AXOS) modulates gut microbiome and its metabolic answer, **Andrea Polo**, (Free University of Bolzano, Italy)
16:25	Food-associated *Lactiplantibacillus plantarum* for the development of innovative non-dairy fermented foods with a beneficial role in ameliorating intestinal inflammation, **Roberta Prete**, (University of Teramo, Italy)
16:35	A dietary supplement based on Mediterranean diet beneficial ingredients modulates gut microbiome composition and activities, **Vincenzo Valentino**, (University Federico II, Napoli, Italy)
16:45	Revitalizing the gut microbiome: unleashing the power of low-sugar fermented juices, **Tlais Ali Zein Alabide** (Free University of Bolzano, Italy)
16:55	NAFLD: behind a statistical approach used for the inspection and association of omics and clinical data, **Francesco Maria Calabrese**, (University Aldo Moro, Bari, Italy)
17:05	End of the scientific programme of the day

18:00

WALKING CITY TOUR

Let's meet in front of the Garibaldi statue

Piazza Garibaldi, 43121 Parma

THURSDAY 28 | September 2023

PAGANINI CONGRESSI
Via Toscana 5/a, 43121 Parma

08:30 | Registration

SESSION 03 | ENVIRONMENT microbiota as a tool for a sustainable future

Chair: Peiying Hong, Monica Agnolucci

09:00	**Plenary lecture: "Building a resilient and sustainable water system for our future urban farms", Peiying Hong, (Kaust, King Abdullah University of Science and Technology, Saudi Arabia)**
10:00	Ecology of soil bacterial communities and evaluation of rhizoremediation potential in a historical polychlorinated biphenyl polluted site, **Sara Borin**, (University of Milano, Italy)
10:20	Molecular and functional diversity of culturable bacterial strains associated with *Tuber borchii* fruit bodies from different Italian sites, **Caterina Cristani**, (University of Pisa, Italy)
10:40	GenBank mining reveals novel clues on rhizobium phylogeny: identical 16S is mostly uncoupled to species name, host plant and country of isolation. How these data suggested the definition of a bacterial h index", **Andrea Squartini**, (University of Padova, Italy)

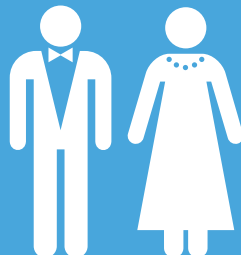
11:00	**COFFE BREAK**
11:15	Poster session
11:45	Sabofleur: isolating and characterising the strawberry flower and bee microbiome for novel and improved microbial control of *Botrytis cinerea*, **Jari Temmermans**, (University of Antwerp, Belgium)
11:55	Fermented wasted bread and brewers' spent grain as next-generation soil amendments: unraveling the interaction with soil microbiota, **Michela Verni**, (University Sapienza, Roma, Italy)
12:05	Metagenome mining reveals how anaerobic and aerobic integrated treatments shape the resistome profile of municipal solid wastes, **Alessandra Fontana**, (University Cattolica del Sacro Cuore, Piacenza, Italy)
12:15	The importance of considering the 'plant microbiome factor' in engineering phytodepuration systems, **Valentina Riva**, (University of Milano, Italy)
12:30	**LUNCH**

SESSION 04 | Exploiting microbiomes for a sustainable future

Chair: Daniele Daffonchio, Olimpia Pepe

14:00	**Keynote: "Ecology of the thermal adaptation of microbial communities in time and space", Daniele Daffonchio, (Kaust, King Abdullah University of Science and Technology, Saudi Arabia)**
14:30	Exploitation of microbial metabolic diversity to support human life in space, **Francesco Canganella**, (University of Tuscia, Italy)
14:50	Microbial consortium adaptation to improve shelf-life, sensorial and nutritional features in sprouted cereals, **Maria De Angelis**, (University Aldo Moro, Bari, Italy)
15:10	Mediterranean spontaneously fermented sausages as a source of lactic acid bacteria for new improved bio-protective cultures and functional starters, **Giulia Tabanelli**, (University of Bologna, Italy)
15:30	**COFFEE BREAK**
15:45	Poster session
16:15	Microbial dynamics and competition in the rhizosheath-root system of xerophytic plants, **Ramona Marasco**, (Kaust, King Abdullah University of Science and Technology, Saudi Arabia)
16:25	*Bacillus haynesii* WVC18 as a sustainable and eco-friendly solution for agricultural application, **Elia Pagliarini**, (University of Bologna, Italy)
16:35	Microrganisms for maize sustainable production: new practices in agriculture, **Maria Elena Antinori**, (University Cattolica del Sacro Cuore, Piacenza, Italy)
16:45	Effect of livestock manure vs digestate as organic fertilizers on bacterial communities of corn silage for dairy cow feed, with a focus on spore-forming bacteria, **Miriam Zago**, (Council for Agricultural Research, CREA Lodi, Italy)
16:55	Wooden shelves: an ancient tool for sustainable cheese ripening in future, **Gabriele Busetta**, (University of Palermo, Italy)
17:05	Group photo
17:30	End of the scientific programme of the day

20:30



GALA DINNER

Palazzo Marchi

Strada della Repubblica 57, 43121 Parma

FRYDAY 29 | September 2023

PAGANINI CONGRESSI
Via Toscana 5/a, 43121 Parma

SESSION 05 | A Sustainable future has come

Chair: Daniele Del Rio, Danilo Ercolini, Erasmo Neviani, Camilla Lazzi

09:00	**keynote, "Research and innovation network on Food and Nutrition Sustainability, Safety and Security: the ONFOODS national partnership" Daniele Del Rio and "The National Center for the Development of New Technologies in Agriculture (Agritech): Microbiota as tools for a sustainable development of agri-food production", Danilo Ercolini, (University of Parma, Italy -University Federico II, Napoli, Italy)**
10:00	TITAN project: Transparency Solutions for Transforming the Food System - Focus on 2 pilots related to microbes, **Antonio Del Casale**, (MICROBION srl, Italy)
10:10	The need of a standard approach in microbiome science: the SUS-MIRRI.IT project, **Maghrebi Sahar**, (University of Torino, Italy)
10:20	Innovative metabolomic and metagenomic approach applied to Parmigiano Reggiano PDO cheese to support traditional features, **Alessia Levante**, (University of Parma and Consorzio del Formaggio Parmigiano Reggiano, Italy)
10:30	MinION sequencing of yeast mock communities to assess the effect of databases and ITS-LSU markers on the reliability of metabarcoding analysis, **Debora Casagrande Pierantoni,** (University of Perugia, Italy)
10:40	**COFFE BREAK**
10:55	Poster session
11:25	Yeasts against grape pathogenic fungi: a sustainable alternative to agrochemicals, **Ileana Vigentini**, (University of Milano, Italy)
11:35	*Hanseniaspora valbyensis*-bioprocessed pomegranate seeds to produce a novel food ingredient, **Fabio Minervini**, (University Aldo Moro, Bari, Italy)
11:45	Exploiting of the agri-food waste and by-products potential to be used as substrate for bioplastic production through *Haloferax mediterranei fermentation*, **Angela Longo,** (Sapienza University of Roma, Italy)
11:55	By-products fermentation: a step forward for the production of new antimicrobial, **Annalisa Ricci**, (University of Parma, Italy)
12:10	Question and answer session of session 5

12:30	Closing ceremony and awards.
12:30	**END OF THE CONFERENCE AND LIGHT LUNCH**
14:00	SIMTREA meeting

SOCIAL EVENTS

WELCOME CEREMONY

TUESDAY 26TH, 6:00 pm

Atrio delle colonne,
Palazzo centrale Università di Parma,
Via Università 12, 43121 Parma

PARMA WALKING TOUR • *prebooked people*

WEDNESDAY 27TH, 6:15 pm

During the Parma city tour we will walk through silent alleys and hidden squares getting to know the city from a local point of view. We will explore the city's most interesting monuments as the Cathedral, the Medieval Baptistery designed by Benedetto Antelami, the Pilotta monument, the Regio Theatre but also the local traditions, people, and styles!

(We will not enter in the monuments)

time: 1,5h of tour

LET'S MEET IN FRONT OF THE GARIBALDI STATUE,
Piazza Garibaldi, 43121 Parma

GALA DINNER • *prebooked people*

THURSDAY 28TH, 8:30 pm
Palazzo Marchi,
Strada della Repubblica 57, 43121 Parma

PLEASE BRING YOUR TICKET

Organizing Committee

Erasmo Neviani (University of Parma, Italy), **Monica Gatti** (University of Parma, Italy), **Camilla Lazzi** (University of Parma, Italy), **Valentina Bernini** (University of Parma, Italy), **Benedetta Bottari** (University of Parma, Italy), **Elena Bancalari** (University of Parma, Italy), **Alessia Levante** (University of Parma, Italy), **Annalisa Ricci** (University of Parma, Italy), **Jasmine Hadj Saadoun** (University of Parma, Italy), **Francesco Martelli** (University of Parma, Italy), **Luca Bettera** (University of Parma, Italy), **Laura Troiani** (University of Parma, Italy), **Saverio Monica** (University of Parma, Italy), **Martina Marrella** (University of Parma, Italy), **Luca Fontechiari** (University of Parma, Italy), **Claudia Della Pina** (University of Parma, Italy), **Gaia Bertani** (University of Parma, Italy), **Laura Marchi** (University of Parma, Italy), **Alessandra Masci** (University of Parma, Italy), **Silvia Zanetti** (University of Parma, Italy), **Teresa Zotta** (University of Basilicata, Italy).

Scientific Committee:

Paul Cotter (Teagasc Food Research Centre, University of Cork, Ireland), **Daniele Daffonchio** (King Abdullah University of Science and Technology, Saudi Arabia), **Daniele Del Rio** (University of Parma, Italy), **Danilo Ercolini** (University of Naples Federico II, Italy), **Marco Gobbetti** (Libera Università di Bolzano, Italy), **Peiying Hong** (King Abdullah University of Science and Technology, Saudi Arabia), **Sarah Lebeer** (University of Antwerp, Belgium), **Erasmo Neviani** (University of Parma, Italy), **Monica Gatti** (University of Parma, Italy), **Camilla Lazzi** (University of Parma, Italy), **Valentina Bernini** (University of Parma, Italy), **Benedetta Bottari** (University of Parma, Italy), **Elena Bancalari** (University of Parma, Italy), **Alessia Levante** (University of Parma, Italy), **Annalisa Ricci** (University of Parma, Italy), **Jasmine Hadj Saadoun** (University of Parma, Italy), **Francesco Martelli** (University of Parma, Italy), **Rosalba Lanciotti** (University of Bologna, Italy), **Luca Cocolin** (University of Turin, Italy), **Carlo Giuseppe Rizzello** (University of Rome, Italy), **Olimpia Pepe** (University of Naples, Italy), **Monica Agnolucci** (University of Pisaa, Italy), **Luca Settanni** (University of Palermo, Italy), **Teresa Zotta** (University of Basilicata, Italy), **Maria De Angelis** (University of Bari, Italy), **Pier Luigi Cardinali** (University of Perugia, Italy), **Cinzia Caggia** (University of Catania, Italy).

Selected lectures
Session 1

FOOD microbiota as a tool for a sustainable future

Twenty years of investigation on riboflavin overproducing food-grade bacteria: biotechnological applications and perspectives

Russo Pasquale[1], Lopez Paloma[2], Del Solar Gloria[2], Requena Teresa[3], Duenas Maria Teresa[4], Aznar Rosa[5], Van Sinderen Douwe[6], Le Blanc Jean Guy[7], Spano Giuseppe[8], Mora Diego[1]

[1] University of Milan Department of Food Environmental and Nutritional Sciences, Milan, Italy
[2] Center for Biological Research Margarita Salas (CIB-CSIC), Madrid, Spain
[3] Institute of Food Science Research(CIAL-CSIC), Madrid, Spain
[4] University of the Basque Country (UPV/EHU), San Sebastian, Spain
[5] University of Valencia, Department of Microbiology and Ecology, Valencia, Spain
[6] University College Cork, Corck, Ireland
[7] CERELA-CONICET, Tucuman, Argentina
[8] University of Foggia, Department of Agriculture, Food, Natural Resources and Engineering (DAFNE), Foggia, Italy

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The food microbiota is an important reservoir of microorganisms capable of increasing the content of essential micronutrients, including B-group vitamins. Riboflavin (vitamin B2) is the precursor of the flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD), coenzymes involved in the electron transport chain and multiple redox reactions. In Gram-positive bacteria, expression of the rib operon for riboflavin biosynthesis is regulated by an FMN-riboswitch consisting of a sensitive domain (aptamer) that, upon binding to the effector (FMN), induces a conformational change in the regulatory domain. Exposure ofvitamin B2-prototrophic strains to roseoflavin (a toxic analogue of riboflavin) allows the selection of spontaneous mutants capable of overproducing riboflavin and harboring mutations in the rib operon riboswitch.

Over the last twenty years, this strategy has allowed the selection of food-grade strains belonging to different species well-known for their technological and/or functional potential. These bacteria are associated with different 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 reverse ariboflavinosis, alleviate intestinal inflammatory diseases, and to provide some anticancer and neuroprotective effects.

Finally, we discuss preliminary results for future efforts, such as i) selection of new strains from spontaneous fermentation and/or unconventional food matrices; ii) optimization of appropriate food processing; iii) elucidation of rib operon regulation; iv) analysis of microbial tolerance to oxidative stress; v) better understanding of the beneficial effect of the consumption of B2-fortified foods and/or B2-overproducing probiotics; vi) exploitation of strategies to alleviate micronutrient malnutrition in developing countries.

The activities are performed in the framework of the ongoing ONFOODS and AGRITECH projects funded by the European Commission – NextGenerationEU (Piano Nazionale di Ripresa e Resilienza).

O1- 2

Back to the complexity: the challenge of natural starter cultures development

Chessa Luigi, Paba Antonio, Daga Elisabetta, Comunian Roberta

Agris Sardegna

Corresponding Author: rcomunian@agrisricerca.it

The biodiversity depletion of the microbiota colonizing the food production environment and the raw material to be processed has led for a long time to the development of selected starter cultures, a convenient solution to carry out the food products fermentation easily and safely. Selected strains, because of their concentration, easily become the dominant microbiota causing a dramatic decrease in microbial diversity and loss of artisanal traditional products' characteristics of typicity. Natural cultures, with an undefined mix of species and strains in equilibrium, reproducible only in their place of origin, contribute to preserve microbial biodiversity and enrich products with sensory features that bind them to their territory. However, their technological performance is not standardized, and their use is not risk-free since, together with useful microorganisms, even pathogen or spoilage ones could be potentially inoculated and allowed to contaminate the product. The aim of this presentation is showing a "new" approach to develop natural starter cultures, potentially applicable at artisanal or industrial scale, guaranteeing safety, quality constancy, technological performances reproducibility, preserving biodiversity and peculiar sensory characteristics usually linked to traditional products, while overcoming the problems associated with the propagation of natural cultures.

Agris Sardegna is investigating the retrieval/development, preservation, reproduction, and use of different complex starters. Complex-microbial-communities collected in the Sixties (*scotta-innesto*), and microbiota colonizing raw ewe's milk or olive brine were investigated to develop starter cultures for Pecorino Romano PDO cheese, artisanal cheeses, and table olives. Strategies for recovering and reproducing biodiversity present in raw matrices or in successfully fermented products were described. Particularly, for table olive better adaptability of autochthonous complex-microbial-communities to fermentation conditions, compared to the two best-performing selected strains, was proved. Recovering, characterisation, and preservation of microbiota and their exploitation should be pursued from the perspective of better sustainability of the environment and its resources.

O1- 3

Dairy environment and seasons affect the microbiome of artisanal cheese

Ferrocino Ilario, Biolcati Federica, Zeppa Giuseppe, Giordano Manuela, Cocolin Luca

Università degli Studi di Torino

Corresponding Author: ilario.ferrocino@unito.it

Cheese microbiome harbored a complex microbial community originating from dairy environment, seasons and raw materials used. In this study, the production chain of Maccagno cheese, a typical product of the Piedmont region (North-West Italy) was monitored and the influence of the dairy environment and seasons in the development of the cheese microbiome was evaluated. Three different productions (during spring, summer and winter) of one dairy plant of Maccagno PDO cheese were considered. Milk, whey starter, curd and cheese at different ripening stages were collected for a total of 151 samples. In addition, 145 environmental swabs such as walls, floors and equipment were collected for metabarcoding and shotgun sequencing. Microbiota compositions was highly influenced by the seasons. *Lactococcus lactis* was mainly associated with autumn, *Lactobacillus delbrueckii* in summer and *Leuconostoc* in winter. In winter, the prevalence of lactic acid bacteria was higher while in summer several spoilages were identified such as *Acinetobacter*, *Citrobacter* and *Enterococcus*. The mycobiota showed the highest prevalence of *Geotrichum candidum* in summer and *Debaryomyces* in winter. The environmental microbiota display in autumn the predominance of *Bacillus* and *Halomonas* while in summer LABs were prevalent. Source tracking analysis was then used to evaluate the microbial transfer from environment to the final product and revealed that metal blade used as mixer in the heater tank was the main microbial source for the final cheese. Indeed, swabs collected from the brine tank and air ventilation system of the ripening room were the source of unwanted fungi that modify the volatile-organic compounds and the metagenomic gene content of the cheese. Due to the importance of dairy environment's microbiome in the quality of final product, plant ecosystem surveillance could become a tool to support cheesemakers.

O1- 4

Dynamics and acidification properties of raw milk bacterial communities during serial fermentations

Gapp Chloé[1], Callon Cécile[2], Mangavel Cécile[3], Theil Sébastien[2], Dijamentiuk Alexis[3], Revol-JunellesAnne-Marie[3], Chassard Christophe[2], Borges Frédéric[3]

[1] Université de Lorraine (LIBio) - INRAE (UMRF)
[2] INRAE (UMRF)
[3] Université de Lorraine (LIBio)

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Microorganisms play a central role in the production of fermented foods. In particular, Lactic Acid Bacteria naturally present in raw milk, or added during cheese making, are essential because of their acidifying action. The traditional production of cheese involves sequential fermentations, or backslopping, where a newproduction is inoculated with part of the previous production. Although this method has been widely used for cheese making, the link between the dynamics of structure and functionality of bacterial communities during this process is not well understood. In this study, twenty-six raw milks sampled in the same cheese PDO geographical area were serially fermented in order to select bacteria adapted to the cheese making conditions. Bacterial community structures and functionality were evaluated during the fermentations by metabarcoding analysis and acidification monitoring. The results revealed that the community structures were highly variable between the different milks and gave rise to different typologies of acidification kinetics succession. Indeed, while the communities exhibited a high structural dynamic, different acidification profiles were observed: stable, progressive or more variable. These results show that there is a very wide range of ecological trajectories for achieving milk acidification. In a larger perspective, this work brings new insights in the field of starter culture design for the inoculation of dairy products.

O1- 5

Variation of microbiota, chemical composition and B-vitamins in milk from alpine pasture and indoor dairy cows

Franciosi Elena[1], Secchi Giorgia[1], Amalfitano Nicolo[2], Bittante Giovanni[2], Perenzoni Daniele[1], Vrhovsek Urska[1]

[1] Fondazione Edmund Mach
[2] Università di Padova

Corresponding Author: elena.franciosi@fmach.it

The aim of this study was to investigate the variation of milk microbiota during and after summer transhumance in relation to milk composition and B-vitamins content. The project involved 26 Italian Simmental cows (5-7 per farm) reared in a 4 permanent farms (PF) located at Levico (Trentino, Italy). The cows were moved in mid-June on 4 summer farms (ALP) located at Passo Vezzena (Trentino) and moved back to PF in September. The animals were monitored and sampled in July and October. Milk microbiology plates count with different culture media; isolation and species characterization by 16S rRNA amplicon; microbiota composition by Miseq Illumina and QIIME2 and B-vitamins content (Thiamine, Riboflavine, Nicotinic acid and Folic acid) were performed for each milk sample. The data were analyzed with a mixed model including the fixed effects of herd, period and their interaction, and the random effects of individual cows nested in the herd and of residual. Identified bacteria taxa were classified into two favourable categories: Pro-Dairy (LAB) and other Probiotics, and two unfavourable categories: Spoilage and Pathogenic bacteria. The results revealed variability among farms, especially one farm had opposite trend in almost all the traits. Milk composition traits mainly showed higher values after the transhumance, and significant interaction among herds for some traits. LAB category has significant different value in PF than ALP. Other Probiotic was higher during ALP. Similar trends were observed in Spoilage and Pathogenic categories. The B-vitamin complex showed higher values, except for Folic acid, during summer pasture than indoor feeding, with small differences among herds. The metagenomics analysis confirmed the high variability of the milk microbiota during and after summer transhumance. Moreover, we observed a large influence of different herd management on milk microbiota variation.

The research was a co-funded PhD byFondazione Edmund Mach and University of Padova.

O1- 6

Microbiome of kefir produced using Amiata donkey milk: characterization of the microbial communities and of fatty acid profile

Daghio Matteo, Toni Elisabetta, Sargentini Clara, Tocci Roberto, Bonelli Antonio, Viti Carlo

University of Florence

Corresponding Author: matteo.daghio@unifi.it

The number of studies on the properties of donkey milk is increasing. Compared to cow milk, donkey milk contains a higher content of polyunsaturated fatty acids (PUFAs) and of lactose. Its characteristics can be further valorised by the production of kefir. In this work Amiata donkey milk was used alone (DK), or in combination with sheep milk (DSK, 50 % each) or cow milk (DCK, 50 % each) for kefir production. Raw milk was fermented 24 h at room temperature and the fatty acid (FA) profile was determined by GC-FID. The microbial communities were characterized by high-throughput sequencing of 16S rRNA gene amplicons(V3-V4 region) at the end of the fermentation and after a 5-day storage (4 °C). An Illumina MiSeq platform was used for the sequencing and DADA2 was used to infer the Amplicon Sequence Variants (ASVs). The composition of the bacterial communities and the FA profile in DK was different compared to the other conditions. A higher content of n3 and n6 PUFAs was observed in DK (9.60 % ± 2.12 % and 22.76 % ± 6.11% of the lipid fraction, respectively) compared to DSK (1.89 % ± 0.11 % and 2.07 % ± 0.12 % of the lipid fraction) and DCK (0.89 % ± 0.28 % and 2.17 % ± 0.29 % of the lipid fraction). The relative abundance of *Lactococcus* at the end of the fermentation was 44.79 % ± 1.31 %, 29.49 % ± 2.37 % and 46.93 % ± 1.67 % in DK, DSK and DCK, respectively. However, the relative abundance of the genus *Lactococcus* decreased to ~1 %, or lower, at the end of the storage. A lower relative abundance of *Lactobacillus* was observed in DK (8.62 % ± 0.23 %) compared to DSK (17.3 % ± 2.38 %) and DCK (20.44 % ± 2.03 %). This work provides for the first time a description of the microbiome in kefir produced using donkey milk.

O1- 7

Characterization of lactic acid bacteria in pursue of potential candidates for starter, adjunct and probiotic cultures

Aspri Maria, Anagnostopoulos Dimitros A., Neophytou Klea, Filippou Grigoris, Bozoudi Despoina, Tsaltas Dimitris, Papademas Photis

Cyprus University of Technology

Corresponding Author: maria.aspri@cut.ac.cy

Lactic Acid Bacteria (LAB) (43 stains) were isolated from fermented goat milk, wine lees and surfaces from winery equipment (grape sorting tables) in order to evaluate their technological and probiotic properties. Diversity of LAB was studied by determination of morphological, cultural, physiological and biochemical characteristics while isolates were identified by phenotypic and molecular techniques.

Molecular identification revealed that the 87.5 % of wine lees samples belonged to *Lactobacillus plantarum* group and *Lactobacillus buchneri*, while 12.5 % of them to the species *Leuconostoc mesenteroides*. At the grape sorting tables 93.5 % of the isolates were identified as *Weissella cibaria* and 6.5 % as *Leuconostoc mesenteroides* while 91 % of the isolates of fermented goat milk samples are *Weissella cibaria*, and 9 % *W. confusa*. All isolates showed limited acidification and proteolytic activities, while no lipolysis was recorded. Moreover, some strains were able to survive the in vitro gastrointestinal simulation conditions and also have inhibitory activities against foodborne pathogens.

Selected lectures
Session 2

HUMAN microbiota as a tool for a sustainable future

O2- 1

Environmental pollution drives adaptation in gut microbiome functions of highly-exposed individuals

De Filippis Francesca, Sequino Giuseppina, Valentino Vincenzo, Ercolini Danilo

Università degli Studi di Napoli Federico II

Corresponding Author: francesca.defilippis@unina.it

The array of all the genes in the microbiome along with the host genes has been defined as the "hologenome". While the host genome is highly conserved, and genetically adapt slowly to the changes in the environment, the microbiome genome can rapidly change in response to the environment, and it has been indicated as a possible additional way of boosting evolution. Humans are daily exposed to a wide range of xenobiotics, that may include different classes of molecules and can reach the gut microbiome directly or can be previously metabolized in the liver. We analyzed gut metagenomes in 359 healthy, Italian adults, grouped according to the level of exposure to environmental pollutants, as well as blood levels of dioxins and heavy metals. Analysis highlighted an increase in gene richness in subjects with a high exposure level (HIGH group). In addition, exposure to different classes of environmental pollutants drives the selection of microbial strains able to degrade these compounds in the human gut. Our results highlighted the role of xenobiotics in shaping gut microbiome composition and activity, suggesting the intriguing possibility that it may be implicated in the host response to environmental pressure.

O2- 2

Development of inhalation powders containing Lactic Acid Bacteria: a way to the lungs to possibly boost respiratory health.

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The existence of a specific lung microbiota opened the way to studies focusing on the correlation between itand respiratory diseases, among which cystic fibrosis (CF). In this disorder, characterized by viscous and adherent mucus in the airways, pathogens can accumulate causing recurrent infections. Protective effects have been shown by the oral supplementation of probiotics such as *Lacticaseibacillus rhamnosus* GG to patients affected by CF, but to date, there are no works addressing the inclusion of health-promoting microbes in inhalable powders directly targeting the lungs. In the present study, we developed inhalation powders characterized by highly viable probiotic/potentially probiotic Lactic Acid Bacteria and by aerodynamic characteristics capable of allowing efficient deposition in the lungs. These powders were demonstrated to have the potential to inhibit clinically relevant pathogens, such as *P. aeruginosa*, without anytoxic effects shown in vitro on human bronchial and lung cellular lines. This suggests a possible supporting role of LAB inhalable powders in contrasting respiratory tract infections. Furthermore, this work represents a promising starting point to explore the modulation of the pulmonary microbiota with the administration ofprobiotic directly to the lung.

O2- 3

Sourdough fermentation supports *in vitro* eubiotic effects of gluten-free bread fortified by insect flour

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Insect powder for food applications has been recently accepted in Europe, but the dietary effects on the human gut microbiome have still to be widely studied in vivo. In this context, personalized gut models may provide robust results for clinical application, reduce time, cost, waste, and animal testing, as the Regulation (EU) 2019/1010 indicates. In this work, a gluten-free (GF) bread fortified with insect protein was considered to gain insights of insect- fortified sourdough bread on the human colon microbiome. Bread prototypes were in vitro tested by coupling Infogest® oro-gastro-duodenal digestion to MICODE colon fermentation. The metabolomics and microbiomics profiles of colonic fermentation of baker's yeast fermented and sourdough-fermented GF breads enriched with insect (*Acheta domesticus*) powder (IP) were compared to control GF breads. The results were two-pronged and indicated that IP-containing GF sourdough breads might improve some general indicators of colonic community eubiotic status but might also bear some potential insults. In details, the GF sourdough with IP was able to; i) increase some beneficial bacteria (*Bifidobacterium spp., Lactobacillus gassseri* and *L. crispatus*); ii) reduce other beneficial bacteria (*Akkermansia muciniphila* and *Bacteroides* spp.); iii) limit the growth of opportunistic bacteria (*Escherichia albertii* and *Clostridium perfringens*) and iii) reduce the growth of sulfurate producers (*Desulfovibrio* and *Bilophila*); iv) produce more organic fatty acids (acetate and butyrate); v) reduce the amount of some harmful compounds (p- cresol); vi) increase speciation of oxidant aldehydes (acetaldehyde and benzeneacetaldehyde). Additionally, acetate, butyrate, and hexanoate were positively expressed in the correlation network plot of insect-enriched breads conversely to controls. These preliminary results carried out by MICODE gut model evidenced that the sourdough process is the best solution to produce IP-fortified GF bread since it supports some potential properties of the final product.

O2- 4

The middle-term intake of hydrolyzed and fermented Arabino Xylan-Oligo Saccharides (AXOS) modulates gut microbiome and its metabolic answer

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As fermentation and hydrolyzation are well-known processes to improve the bioavailability of nutrients and enable the fortification with dietary fibers, the effect of such pretreatments on the prebiotic features of ArabinoXylan-OligoSaccharides (AXOS) was explored. The middle-term *in vitro* simulation through the Simulator of the Human Intestinal Microbial Ecosystem (SHIME) demonstrated that the feeding with different formulations (namely oat bran, rye bran and wheat bran) containing hydrolyzed AXOS fermented by lactic acid bacteria significantly increased the synthesis of short-chain fatty acids (SCFA) by colon microbiota. After two weeks from the intake interruption (wash out period), SCFA concentrations significantly decreased but remained still significantly higher compared to the original condition. The microbiome showed a significant reshape, with an abundance increase in Lactobacillaceae taxon after feeding with all fermented and hydrolyzed formulates. The fungal community was also modulated after feeding, with an increase in *Candida* relative abundance and a decrease in *Issatchenkia*. On the contrary, the intake of non-hydrolyzed and non-fermented control did not produce relevant changes of relative abundances. After two weeks from intake interruption such changes were mitigated, and the gut microbiome modulated again to a final structure that was more like the original condition. This finding suggests that hydrolyzed AXOS fermented by lactic acid bacteria could have a more powerful prebiotic effect compared to non-hydrolyzed and non-fermented control, shaping the colon microbiome and its metabolic answer.
However, the intake should be continuous to assure persistent effects. Opening a window into the ecologicalevolutions and plausible underlying mechanisms, the findings reinforce the perspective to explore more in depth the use of hydrolyzed and fermented AXOS as additional ingredient for food fortification.

O2- 5

Food-associated *Lactiplantibacillus plantarum* for the developmentof innovative non-dairy fermented foods with a beneficial role in ameliorating intestinal inflammation

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Fermented foods have regained popularity in Western diets, for their health-promoting potential, mainly related to the role of Lactic Acid Bacteria during the fermentation process. Among them, *Lactiplantibacillus (Lpb.) plantarum* strains, widely used as starter cultures in the production of fermented foods, showed the potential to affect host health. Our studies aimed to investigate selected *Lpb. plantarum* strains, isolated fromfermented foods, to face oxidative stress and related inflammatory damage at intestinal level and their application as multifunctional starter cultures to produce innovative non-dairy fermented foods with anti- inflammatory properties. For these purposes, the specific ability of each strain to modulate ROS levels in response to either oxidative or inflammatory stress and to restore inflammation *via* IL17/IL23 axis in an inflamed intestinal cell model was investigated. Subsequently, two selected strains have been applied in a lab-scale fermentation to produce fermented beverages using cereal and legume flours. Thus, we examined their antioxidant potential and their ability to modulate the expression of pro- and anti-inflammatory cytokines (IL8, IL10) and to trigger IL12-IL17/IL23 axis at intestinal level, a pro-inflammatory pathway considered a promising therapeutic target for its involvement in the pathophysiology of chronic intestinal inflammatory diseases. In addition, the anti-inflammatory effect of *Lpb. plantarum* together with a biologically debittered olive pomace have been confirmed in a mouse model of dextran sodium sulfate (DSS)-induced chronic colitis. The simultaneous oral administration of a diet enriched with fermented olive pomace and a *Lpb. plantarum* strain patented as probiotic significantly improve the macroscopic and microscopic colitis scores with a significantly reduction of the expression of inflammatory and pro-fibrotic cytokines. Our results highlight the potential beneficial contribution of fermented foods through their microbial components (as *Lpb. plantarum* species) and their suitable application in non-dairy products fermentation to develop vegetable-based fermented foods as an alternative strategy to ameliorate intestinal inflammation.

O2- 6

A dietary supplement based on Mediterranean diet beneficial ingredients modulates gut microbiome composition and activities

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The Mediterranean diet (MD) is a plant-based nutritional pattern that is high in polyphenols with anti- inflammatory and antioxidant activities. These molecules, combined with fibers, can modulate the composition of the human gut microbiome, leading to the selection of beneficial microbes that secrete various health-promoting metabolites such as short-chain fatty acids (SCFA) and urolithins. To further investigate this potential, a double-part study was conducted to test a superfood that simulates the composition of the MD.
In the first part of the study, the superfood or a placebo was fed to the Simulator of Human Intestinal Microbial Ecosystem (SHIME®) for 14 days. The microbiome of both lumen and mucosa samples was evaluated at baseline, 7 and 14 days of treatment, as well as after a 7-days of wash-out period. Results showed that treatment with the superfood enriched both the lumen and mucosa with species such as *Ruminococcus* and *Faecalibacterium*, which are health-related metabolites producers, as well as with geneslinked with the production of SCFA, isothiocyanate, and urolithins. Furthermore, analysis of Carbohydrate- Active Enzymes (CAZy) highlighted that the superfood enhanced the microbial potential to degrade complex fibre.
In the second part of the study, a dietary supplementation with the superfood for 2 months was carried out on 122 overweight subjects. Preliminary results showed a selection of fibre-degrading taxa in treated subjects compared to the placebo group, as well as an increased abundance of genes linked with the production of SCFA, including butyrate.
These results demonstrate the potential of the MD-based superfood to modulate the gut microbiome byselecting beneficial taxa with health-promoting metabolic traits.

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O2- 7

Revitalizing the gut microbiome: unleashing the power of low-sugar fermented juices

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Notably, a rise in the prevalence of these disorders has been vigorously linked to excessive sugar consumption. A high-sugar diet causes changes in the gut microbiome (dysbiosis) by favoring the structure of a microbiota associated with an obese phenotype. Additionally, a high-sugar diet disrupts of the intestinal barrier, which induces the production of the pro-inflammatory cytokines IL-6, IL-1?, and TNF?, leading to intestinal inflammation. The consumption of natural fruit juices, valid substitutes for fresh fruits, results into an unconscious excessive intake of free sugars. Our study aimed to design a biotechnological fermentation process (single and sequential fermentation) that exploits the metabolic potential of lactic acid bacteria and yeasts to create fruit juices with a reduced sugar content (ranging from 28 to 68 % reduction). Thereafter, the impact of low sugar fermented juices on the composition and functionality of the gut microbiota was assessed through in vitro Simulator of the Human Intestinal Microbial Ecosystem. Feeding with various juices significantly altered the microbial community composition in both the distal and proximal colon, as indicated by the Bray-Curtis dissimilarity analysis. Additionally, stipulated that gut microbiota plays an important role in maintaining the health and function of the intestinal barrier, the efficacy of low-sugar fermented juices on barriers integrity, cytokines, mucin and reactive oxygen species release, and glucose intake was in vitro evaluated using Caco-2 cells. Fermented juices, particularly FJY and FJSeq, exhibited the highest protective effects against inflammatory processes and oxidative stress in Caco-2 cells. We believe our findings can contribute to shed light on the mechanisms underlying the complex relationship between dietary habits, gut microbiota and human health.

O2- 8

NAFLD: behind a statistical approach used for the inspection andassociation of omics and clinical data

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Non-alcoholic fatty liver disease (NAFLD) prevalence and its related comorbidities are rapidly increasing and, if maintaining the current growth rate, they will reach epidemic proportions in the next coming few years. On the clinical point of view, several pathologic traits of NAFLD are shared with obesity, but others are closely linked to specific altered functions, proper of the symptomatic framework featuring the microbiota gut-brain axis of NAFLD. The altered homeostasis status revealed a burden of variables that we need to study to obtain a more complete clinical picture. In our advanced analysis workflow, the knowledge of gut microbiota members nearly inhabiting the gut niche, is granted by an approach that merges two distinct but converging omics i.e., faecal metabolomics coupled with metabarcoding taxa annotation. As a result, volatile organic compounds and 16S rRNA taxa signatures were used to ascertain the impact of dietary lifestyle and physical activity on a cohort of 109 NAFLD patients, randomly allocated to six lifestyleintervention groups, where Mediterranean diet, aerobic and anaerobic activity were combined. Moreover, being us interested in inspecting other stratifications, the synergistic effect of diet and physical activity were also tested against a cohort subgroup that followed a treatment based on physical activity alone. The positivecombined treatment evidently agrees with the decreased 'controlled attenuation parameter' (CAP) value, indicative of hepatic steatosis level. Additionally, a stringent statistical approach allowed the selection of those biochemical clinical parameters that we gathered into four factors, based on rotating factor analysis and namely: i) inflammatory/sub-inflammatory, ii) a metabolic factor based on glucose, HbA1c, C peptide, insulin, (iii) a parenchymatous/hepatic factor and iv) a factor in common with cardiovascular pathologies (total and direct bilirubin and haemoglobin). Downstream, statistical correlation of VOC, taxa and clinical variables, allowed us to statistically weight each detected variables with a biological rationale.

Selected lectures
Session 3

ENVIRONMENT microbiota as a tool for a sustainable future

Ecology of soil bacterial communities and evaluation of rhizoremediation potential in a historical polychlorinated biphenylpolluted site

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The Site of National Priority (SIN) Brescia-Caffaro is a highly polluted area in Northern Italy presenting a historical mixed and uneven contamination by metals and organic pollutants, in particular polychlorinated biphenyls (PCBs), often exceeding the legal thresholds. This site represents a model to study soil microbial ecosystems and design sustainable solutions aimed at the remediation of large, aged-contaminated areas. Thechemical and microbial profiling of the soils in the SIN former agricultural areas demonstrated that pollutants were among the main drivers of soil microbiome assembly. The widespread detection of degradation potential in topsoil layers, where we retrieved the higher pollution level, indicated that the autochthonous bacterial communities could be exploited in soil reclamation strategies based on plant biostimulation.

A rhizoremediation approach was therefore explored by testing at greenhouse level different combinations of plant species and soil treatments. After 18 months, the higher decrease of the original PCB congener concentration was obtained planting *Phalaris arundinacea* L., subjected or not to soil periodic flooding to induce a redox cycle compatible with both reductive dichlorination and PCB aerobic degradation. We then applied stable isotope probing (SIP) using 13C-labeled 4-chlorobiphenyl (4-CB) and 16S rRNA amplicon sequencing on the biostimulated soil, to determine how the structure of total bacterial community was affected by the different treatments and to identify the bacterial populations actively involved in PCB degradation. The most abundant taxa deriving carbon from 4-CB were Betaproteobacteria and Actinobacteria. Comamonadaceae was the family most represented in planted soils, Rhodocyclaceae and Nocardiaceae in non-planted soils. Planted soils subjected to redox cycle enriched PCB degraders affiliated to Pseudonocardiaceae, Micromonosporaceae and Nocardioidaceae. Overall, results showed different responses of bacterial taxa to specific rhizoremediation treatments and provided new insights into those active in PCB biodegradation.

O3- 2

Molecular and functional diversity of culturable bacterial strains associated with *Tuber borchii* fruit bodies from different Italian sites

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Tuber borchii is an ectomycorrhizal fungus establishing important beneficial symbiosis with the roots of several tree species from different botanical families. *Tuber borchii* fruit bodies are associated with diverse bacterial communities, characterized by plant growth promoting (PGP) abilities, such as the production of compounds capable of contrasting the growth of common soil-inhabiting phytopathogens. In this work we studied the molecular and functional diversity of bacterial strains, referable to *Pseudomonas fluorescens*, selectively isolated from *Tuber borchii* samples, collected in three distinct Tuscany geographic areas with high truffle vocation. To this aim, 120 fluorescent colonies were randomly selected and isolated on King's B agar, molecular characterized by Amplified Ribosomal DNA Restriction Analysis and then identified by amplicon sequencing of the 16S rRNA gene and two housekeeping genes, namely gyrase subunit B (gyrB) and RNA polymerase sigma factor (rpoD). Most of them were affiliated to the *Pseudomonas fluorescens* group and some isolates to *Paenibacillus taiwanensis*. Such isolates were functionally characterized assessing their ability to produce siderophores, using the universal Chrome Azurol Sulphonate (CAS) assay. The results obtained showed that siderophores producing isolates were 81,4 % of the total, as revealed by a visible halo on CAS agar. Actually, 42.4% produced a discoloration halo from 0.3 to 0.8 cm and 27.9 % from 0.9 to 1.4 %. Finally, 11 % accumulated the higher amounts, producing discoloration halos larger than 1.5 cm.In conclusion, our results allow us to identify functional bacterial strains to be used as specific bacterial inocula for both biocontrol, plant growth promotion and improvement of fungal performance and mycorrhizal establishment.

O3- 3

GenBank mining reveals novel clues on *Rhizobium* phylogeny: identical 16S is mostly uncoupled to species name, host plant andcountry of isolation. How these data suggested the definition of a“bacterial h index”

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We devised an in-silico strategy, of which we present the proof of principle, to infer a series of ecological and evolutionary pieces of information over a given microbial taxon using public database sequence records. The starting assumption was that, if a *Rhizobium* 16S sequence would display 100 % nucleotide identity with a number of other GenBank subjects, they should represent a group of close phylogenetical kins. But given such kinship, to what extent would such cases share also host and geography. Upon downloading records and using R to pick the requested homologies we visualized the percentages of cases that, besides the 100 % nucleotide identity, would share also one feature out of the following three metadata: a) species name; b) host plant, c) country of isolation, or two of them, or all of them, or none. Among several novel evidences, one striking observation was that in more than 65 % of the cases, the three descriptors were all different. This suggests that, although a certain rhizobium is phylogenetically indistinguishable from all its database companions, there is very little constraint to maintain a common host plant or to remain in a given geographical site.

A byproduct of this analysis, that can apply to any taxon, was to realize the possibility of treating data as ifthey were 'citations'. A single sequence scoring positive could ‘pick’ from one to several hundreds of identical matches in GenBank. The more we find the same query sequence, the more we can consider it ‘cited’, i.e. re-proposed in the world, Thus one can also analyze the h-index of the ranking: (those identicalphylotypes were all 'produced’ by the *Rhizobium* ‘author'). In our score, we found a *Rhizobium* h index = 201 as the query in the 201st position has found 202 identical homologues in the database.

Sabofleur: isolating and characterizing the strawberry flower andbee microbiome for novel and improved microbial control of *Botrytis cinerea*

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The fungus *Botrytis cinerea*, the causal agent of grey mould, is the most common cause of strawberry fruit loss. Despite agriculture's dependence on chemically synthesized plant protection products, pathogens acquiring resistance and increased environmental concerns, have increased the need for alternatives. One alternative is biocontrol, a strategy assumed to have less impact on the environment with pathogens being less likely to acquire resistance due to the multifactorial mode of action of the biocontrol agent. One of the major hurdles of biocontrol is their inconsistent field performance, as biocontrol agents (BCAs) are often not adapted to this stressful environment and have a low survival rate. Primarily strawberry flowers are vulnerable organs. A thorough understanding of the biotic and abiotic stressors and the ephemeral microbial communities of these habitats, including the closely related bee habitat is paramount in biocontrol. In our novel citizen science project called 'Sabofleur', we aim to isolate and identify potential BCAs from strawberry flowers of 50 professional cultivators in Flanders and Estonia. Particularly, Lactic Acid Bacteria (LAB) are considered, due to their high antipathogenic activity, food-grade application and the knowledge gap concerning their occurrence and application on plants such as strawberry. So far, more than 20 LAB strains with new and relevant adaptation and biocontrol properties were isolated. One isolate reduced Botrytis growth by more than 80% in plate assays, surpassing commercial *Pantoea agglomerans*. Subsequently, Sabofleur provides novel fundamental knowledge on the ecology of LAB on flowers and pollinators. Using metagenomic sequencing, the effects of cultivation type and pesticidal use on the strawberry flower microbiome are being assessed. Furthermore, the contact microbiome between strawberry flowers and pollinator *Apis mellifera* was determined in a commercial organic field. By sampling flowers and pollinators from the field and the hives, the importance of pollination for the plant's microbiome is estimated.

O3- 5

Fermented wasted bread and brewers' spent grain as next-generation soil amendments: unraveling the interaction with soil microbiota

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The application of novel soil amendments and the exploitation of plant growth-promoting microorganisms are considered promising tools for developing a more sustainable agriculture in times when ensuring high-yield production with limited resources is essential.
In this study, wasted bread and brewers' spent grain, native and fermented with selected strains of *Lactiplantibacillus plantarum*, were used as soil amendments during 8weeks-long pot trial. An integrated approach was used for assessing the modification of the physicochemical properties of a typical Mediterranean alkaline agricultural soil, the plant growth-promoting effect on tomato plants (*Solanum lycopersicum* L.), as well as the interaction with soil microbiota through culture dependent and independentanalysis.
Amendments raised organic carbon and total nitrogen content in soils; the lower pH and the higher organic acid content, compared to unamended pots, also determined a major availability of micronutrients and positively affected plant growth. Isolation from soils showed an absence of cultivable lactic acid bacteria after the first week of trial, yet a preliminary study, using a combined DNA&RNA extraction from soil followed by a metabarcoding and targeted gene expression assays, revealed the presence of several fungal species, both symbiotic and pathogenic. Nevertheless, the latter were not vital when soils were supplemented with fermented amendments, suggesting that although starters used for fermentation might not have withstood soil conditions, the changes induced in soil microbiota and composition persisted throughout the cultivation period.
The results collected so far, not only encourage the application, for agricultural purposes, of food industry side-streams subjected to fermentation, but also provide new insight on the interaction of fermented biomasses with soil microbiota.

Metagenome mining reveals how anaerobic and aerobic integratedtreatments shape the resistome profile of municipal solid wastes

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Nowadays, the management of municipal solid wastes poses a significant challenge. To address this issue, anaerobic digestion and aerobic composting processes have emerged as the most promising methods for treating the organic fraction of municipal solid waste. These processes rely on complex communities of microorganisms and result in the production of biogas and compostable substances that have applications inagriculture. Furthermore, these integrated approaches have the potential to mitigate the dissemination of antibiotic resistance genes, as municipal solid waste serves as a significant reservoir of such concerning genes.

The objective of this study was to conduct a comprehensive analysis of the microbiome in an integrated facility that combines anaerobic digestion of the organic fraction of municipal solid waste with composting of the resulting solid digestate and green wastes. To achieve this, the DNA shotgun sequencing technique was employed to unravel both the taxonomic composition and functional profiles of the microbial community, with a specific focus on the presence of antibiotic resistance genes (referred to as the resistome). The findings revealed that the integrated treatment significantly influenced the microbiome, with the species *Hyphomicrobium denitrificans*, *Sphaerobacter thermophilus*, and *Thermobifida fusca*, as well as genes related to the two-component regulatory system for nitrogen metabolism, being the primary discriminant features during the composting phase. Additionally, the resistome exhibited compositional changes at various stages of the plant's operation. Notably, composting exerted the greatest impact on the abundance of antibiotic resistance genes, particularly those associated with drug classes such as tetracycline and fluoroquinolones.

O3- 7

The importance of considering the 'plant microbiome factor' inengineering phytodepuration systems

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Constructed wetland (CW) systems are eco-friendly and cost-effective technologies for water reclamation, where the plant holobiont play a fundamental role, improving pollutants removal and maintaining the stability of the system. Nonetheless, scientific research on phytodepuration mostly focuses on engineering aspects, while plants and their associated microbiome are generally neglected.
Here, we investigated the bacterial communities associated to the root systems of *Phragmites australis* and *Typha domingensis*, collected from a CW receiving a mix of primary treated agricultural, municipal and industrial wastewaters. We established a collection of 160 endophytic bacteria looking for strains exploitable for microbial assisted phytodepuration. The taxonomic identification of the isolates revealed a sharp differentiation between the collections obtained from *P. australis* and *T. domingensis*, mainly represented by lactic acid bacteria (98 %) and Enterobacteriaceae (72 %), respectively. Apparently, these two plant species, thriving in CW under the same growing conditions and receiving the same wastewater, can recruit different bacterial communities in the root systems. We decided to further investigate the microbiome composition of the root endosphere and three root system fractions collected at increasing distance from roots surface, applying high-throughput 16S rRNA amplicon sequencing. Both the fraction and the plant species were recognized as drivers of the bacterial community structure. Moreover, in all the fractions, several bacteria families were significantly enriched in *P. australis* or in *T. domingensis*, confirming the hints provided by ASV phylogenetic affiliation. The results obtained on the culturable root-associated bacteria did not allow us to exploit them as biotechnological tool for bioaugmentation in CWs, while the results on bacterial diversity clearly showed that macrophytes can recruit specific bacterial communities in the root system. We suggest the importance of systematically considering the plant in relation to the associated microbiome, beside engineering aspects, to select the most suitable species when designing phytodepuration systems for specific wastewaters treatments.

Selected lectures
Session 4

Exploiting microbiomes for a sustainable future

Exploitation of microbial metabolic diversity to support human lifein space

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Traveling to deep space is difficult for many reasons, and food is a crucial one. Round-trip Mars mission scenarios last 3 years, demanding food with a shelf-life of 5 years. This means that feeding human crew sustainably for long-duration missions will lead to different approaches from the current Earth-based food production systems. The cost of launching food into space is very high. The only alternative is to make food during missions using methods such as artificial light photosynthesis, greenhouses, nonbiological synthesis of food, microbially-generated electricity, etc.. Among the most investigated alternatives, growing crops and microalgae single cell protein (SCP) using artificial light photosynthesis, have been explored. Because Earth-based food safety systems cannot be directly applied in space, safety assurance is also a critical bottleneck in the space production of food and other bioproducts. Future sustainable deep-space missions will then require to devote more resources to understand the biological and physical science principles underlying microbial food safety in space. Microbial cultures may be used to produce not only familiar fermented foods, but also essential nutritional supplements and pharmaceuticals for crew health. Other related future applications of microorganisms may include probiotic supplements, plant-promoting bacteria, farming of microalgae, and even lab-grown meat, all of which will require novel food safety protocols. As an example, newly isolated strains of bacteria aboard the International Space Station (ISS) belonging to the family Methylobacteriaceae, were recently reported as possible candidates for plant growth support in space. Methylobacteria are in fact species often involved in important plant processes like nitrogen fixation, phosphate solubilization and abiotic stress tolerance. Concerning the potential use of agrofood microorganisms in space, human health, and biosafety inside the ISS, we have been performing experimental studies about survival of bacterial strains, human microbiota and biofilms aboard the ISS, as well as in confined space simulations.

O4- 2

Microbial consortium adaptation to improve shelf-life, sensorialand nutritional features in sprouted cereals

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Germination is a traditional process helpful in obtaining new plants from grains. Literature evidence agrees in recognizing a nutritional value improvement supported by the addition of sprouted cereals and legumes. Hence, the sprouting process would lead to physiological and biochemical changes that in turn would affect the presence and abundance of microbiota taxa inhabiting this ecological niche. With the aim of testing different compositions and dough yield conditions, we inspected the metatranscriptomics and metabolomics profile in different fermented cereals made of refined commercial wheat flour, sprouted and non-sprouted whole wheat flour, and a blend composed of sprouted whole wheat and sprouted lentils. As evidently supported by the inspection of transcripts and volatile organic compounds from in-double analysed samples, the microbiota was shaped by both the sprouted process and the dough yield condition.
Based on high-resolution metabolomics and metatranscriptomics merged results we were confident to detect statistically significant altered transcripts at the species level, together with related metabolites. When sampling groups are compared, the most different metabolic subpathways belonged to carbon (starch/sucrose/galactose metabolism, pentose phosphate pathway, pentose glucuronate interconversion) andnitrogen (arginine/lysine/glicine) metabolisms, as well as cell-cell communication pathways (two- component system and quorum sensing). Downstream to the evaluation of all significant differences in metabolic activities, we were able to undoubtedly detect an improved nutritional quality and related shelf- life in the whole wheat and mixed grain sprouted samples. The present omics results are a powerful tool for the selection of those technological traits that are of fundamental importance in the reduction or decrease of food loss. Nowadays, cereals constitute 19 % of the volume of all wasted foods and we need to lower this proportion in the perspective of a more sustainable future where the managing of food chains will take the scene.

O4- 3

Mediterranean spontaneously fermented sausages as a source of Lactic Acid Bacteria for new improved bio-protective cultures andfunctional starters

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Global projections show that food demand will increase by 35 % by 2030. In this perspective, waste reduction in production systems through prolongation of food shelf life and enhancement of food safety can become a global strategy to guarantee the environmental sustainability of agro-food systems. These goals can be achieved thought an optimization of biotechnological tools and the development of sustainable approaches, affordable also by local producers in small-scale production where food safety can be a recurrent concern.

This work, part of the H2020 PRIMA BioProMedFood project, is aimed to valorize the biodiversity and genetic bacterial heritage represented by traditional meat products of the Mediterranean area, through isolation and selection of autochthonous bio-protective Lactic Acid Bacteria (LAB) strains with relevant technological characteristics. These LABs have been studied for their safety, antimicrobial activity and technological traits to be exploited as functional starters or bioprotective cultures in meat products, reducingsafety risks.

In particular, more than 2000 isolates were obtained, and the resulting 325 different biotypes were genetically identified and tested for their safety (biogenic amines and antibiotic resistance) and bio-protective activities against some foodborne pathogens and spoilage bacteria. The 30 most promising biotypes were further technologically characterized to select the best LAB candidates to be studied as bioprotective cultures in fresh sausages and tailored starters for traditional fermented sausages, highlighting interesting anti-listeria potential and good technological properties. In addition, some strains were able to confer peculiar organoleptic profiles to the products, confirming the role of these autochthonous cultures in the recognizability of the product variety of this important food sector. The application of new LAB cultures provided innovative biotechnological tools for the valorization of food natural microbiota still present in the Mediterranean basin, addressing some important challenges for a safer Mediterranean meat supply chains and a sustainable use of resources.

Microbial dynamics and competition in the rhizosheath-root system of xerophytic plants

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In (hyper)arid-desert ecosystems, where resources are limited, xerophytic plants have evolved unique morphological and physiological adaptations to optimise water and nutrient uptake and storage. One of such adaptations is the rhizosheath root system, which acts as a “mini-oasis” with enriching resources in otherwise nutrient-poor soils. This resource-rich environment not only benefits the plant but also attracts a rich animal biota and a diverse edaphic microbiota, including bacteria, archaea, and fungi. However, according to the Darwinian “Survival of the Fittest” theory, one can expect intense competition among desert soil microorganisms to colonise such favourable niche: only microorganisms equipped to sustain their own survival and favour the host plant’s fitness through biopromotion and biofertilisation activities, can succeed. By examining the metabolic properties from microbiome metagenomes of rhizosheath-root speargrasses from different deserts, we demonstrate that edaphic microbial community diversity, stability and biomass increased from the non-vegetated soils to the rhizosheath–root system. The microbial communities in non-vegetated soils predominantly exhibited an autotrophic lifestyle, while those associated with the plant niches were primarily composed of heterotrophs and enriched in microbial plant-growth-promoting traits, antibiotic resistance genes and CRISPR-Cas motifs, as also corroborated by the cultivation and screening of beneficial bacteria involved in exopolysaccharide production and hormone biosynthesis. These results support the hypothesis that the colonisation of the rhizosheath—that is a favourable niche—triggers an intense microbial “Arms Race” able to control the microbial biomass and promotes the selection mediated by plants of microorganisms beneficial to the fitness and resilience of the host in a win-win interaction. By applying Darwin’s evolutionary theory, we further postulate that these dense and competitive niches may also represent evolutionary hotspots that can select specific microorganisms offering new adaptive strategies for the holobiont homeostasis in extremely harsh and inconsistent environmental conditions of deserts, including those arising from climate change scenarios.

O4- 5

Bacillus haynesii WVC18 as a sustainable and eco-friendly solutionfor agricultural application

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An increasing need for a more sustainable agriculturally-productive system is required in order to preserve soil fertility and reduce soil biodiversity loss. The Farm2Fork Strategy, published in May 2020 by the European Union, supports the transition to a more sustainable, productive system in which the dependency on pesticides, antimicrobials, and over-fertilization will be drastically reduced. The rhizosphere and its microbial community have a central role in the development of healthy relations with soil and plants.
Members of *Bacillus* spp. have been widely used to enrich soil/root interface to provide plant growth promoting activities. A new isolate, *Bacillus haynesii* VWC18 (Patent N°102023000006816) has been tested in greenhouse, in lettuce (*Lactuca sativa* L.) pots at different concentrations and application time (single and multiple inoculum) to evaluate the best application mode. Analysis of foliar fresh/dry weight and nutrient uptake evidenced a significant response of all applications. The lowest and the highest doses, applied every ten days until harvest, were the most effective; the nutrients yield (N, K, P, Na, Ca, Fe, Mg, Mn, Cu and B) increased more than twice. A new randomized block design with three replicates was then performed in lettuce and basil (*Ocinum basilicum* L.) with the two best performing concentrations applied every ten days. In addition to previous analyses, roots weight and photosynthetic pigments concentration were also examined. Both experiments confirmed the efficacy of the inoculum; the administration of *Bacillus haynesii* VWC18 promoted plant growth, chlorophyll synthesis and mineral uptake in both crop species. Roots weight duplicated or triplicated compared to control plants; the chlorophyll concentration increased as well. Both parameters showed a dose-dependent increase. *B. haynesii* WVC18 is a promising biological tool to improve nutrients bio-availability and increase crop yield.

O4- 6

Microorganisms for maize sustainable production: new practices in agriculture

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Agriculture is primarily affected by climate change, which requests to reduce GHGs emissions and challenges crop production through drought, temperature increase and reduction of biodiversity above all. Inthe last years, different solutions came to these issues, from regenerative agriculture to the application of Plant Growth Promoting Microorganisms (PGPMs), which could replace traditional practices as fertilization and irrigation. However, these practices are seldom considered together. This work, realized and financed in the framework of PRIMA project SIRAM (Sustainable Innovations for Regenerative Agriculture in the Mediterranean Area), focuses on the application of no tillage plus cover crops, vermicompost fertilization and PGPM application as strategies to foster maize sustainable cultivation in Emilia-Romagna region (Italy).

More than 100 bacteria and fungi were isolated from maize rhizosphere and screened for PGP activities in vitro and in pot. In particular, IAA and siderophores production, ACC deaminase activity, phosphate solubilization, nitrogen fixation and drought resistance were evaluated *in vitro*, also in terms of effect on seed germination and seedlings development. Bacteria of genera *Pseudomonas*, *Nesterenkonia*, *Burkholderia* and *Bacillus* and fungi of genus *Trichoderma* were selected and combined in three distinct consortia.

Different formulations (seed coating and vermicompost inoculum) for selected PGPM were tested both in vitro and in pot in terms of efficacy and shelf-life to find the optimal formula to apply in field. Moreover, thecombined action of PGPM and vermicompost fertilization was measured in pot in comparison with standard chemical fertilization. Preliminary results showed that PGPM inoculum were best effective in combination with vermicompost and affected root development since the first stages of seedling development.

Simultaneous application of regenerative agriculture strategies and PGPM not only enhances plant yield and nutritional status, thus allowing maize sustainable production in a context of climate change, but also supplies innovative formulas for the application of microbial biofertilizers.

O4- 7

Effect of livestock manure vs digestate as organic fertilizers on bacterial communities of corn silage for dairy cow feed, with a focuson spore-forming bacteria.

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An increased recycling of nutrients from organic waste to support feed and food production is important for achieving sustainability since organic fertilizers provide nutrients and positively affect the soil microbiota. In the Po valley, the widest cultivated crop to feed dairy cows is corn for silage, whose quality also depends on the number of bacterial spores, that directly affects the spore contamination of milk, related to the risk of late- blowing defect in long-ripened hard-cheeses. The aim of this study was to explore the bacterial community along the production and use of corn silage from fields treated with different organic fertilizers, i.e. cow manure (CM) vs. digestate (DG). The evaluation of microbial communities, focusing on spore-forming bacteria, was performed on samples of soil, chopped corn, corn silage, total mixed ration (TMR), bedding material and milk, with metabarcoding and LH-PCR technique. Metabarcoding and LH-PCR data showed a distribution of spore-forming bacteria more related to the type of samples than to the fertilization treatments, with a relative abundance of *Paenibacillus* and *Bacillus* in silage and TMR samples, and of clostridia in bedding material and milk samples. The results of this study did not reveal a specific effect of the type of treatment (i.e. organic fertilization with CM vs DG) on the spore- forming bacterial communities in the field-to-milk supply chain. However, an influence on the composition of microbiota of various matrices linked to the animal feed cycle until milk was highlighted. Overall, some variations in the microbial communities also occurred. Metabarcoding data showed an abundance of genus *Acetobacter* in chopped corn and silage, and of genera *Weissella* and *Staphylococcus* in bedding material, for CM vs. DG samples, respectively, whereas milk samples from CM chain showed an abundance of genus *Pseudomonas* compared to *Staphylococcus* and *Streptococcus* genera in DG chain samples.

O4- 8

Wooden shelves: an ancient tool for sustainable cheese ripening in future

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The rind acts as a protective barrier for internally-bacterial ripened cheeses. Unlike surface-inoculated smear cheeses, centripetal maturation is not assumed to occur in these cheeses. This research was aimed to evaluate the microbial diversity of the wooden shelves used for the ripening of Protected Denomination of Origin (PDO) Pecorino di Filiano and Protected Geographical Indication (PGI) Canestrato di Moliterno cheeses. The microorganisms associated with the rind of these cheeses were also investigated. Both wooden shelf surfaces and cheese rinds were sampled by brushing method to collect their biofilms. Wooden shelves showed levels of total mesophilic microorganisms (TMM) between 5.6 and 7.2 Log CFU/cm^2, while cheese rinds between 6.1 and 8.8 Log CFU/cm^2. The major dairy pathogens were never detected, while mesophilic and thermophilic coccus Lactic Acid Bacteria (LAB) dominated the surfaces of all wooden shelves and cheese rinds. LAB community was dominated by *Enterococcus* sp., *Leuconostoc* sp., and *Marinilactibacillus* sp. Among yeasts, *Debaryomyces* sp., *Candida* sp., and *Talaromyces* sp. were identified, while *Aspergillus* sp., and *Penicillium* sp., dominated the community of filamentous fungi. MiSeq Illumina analysis identified 15 phyla, 13 classes, 28 orders, 54 families, and 56 genera. *Staphylococcus* sp. was identified from all wooden surfaces, with a maximum abundance of 71 %. *Brevibacterium*, *Corynebacterium* and halophilic bacteria were detected in almost all samples. This study confirmed that the wooden shelves used for cheese ripening are microbiologically active and represent safe systems. Furthermore, the results of this work clarified the transfer flow between wooden shelves and PDO Pecorino di Filiano and PGI Canestrato di Moliterno cheese surfaces: wooden shelves mainly transferred smear-active bacteria to cheese rind, contributing to the development of the final organoleptic characteristics, while cheeses transferred LAB defining for the safety aspects of the shelves.

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Selected lectures
Session 5

A sustainable future has come

TITAN project: Transparency Solutions for Transforming the FoodSystem - Focus on 2 pilots related to microbes

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Transparency is a critical component in modern food systems. Indeed, from an authentic Farm2Fork perspective, it is the key for linking all the actors of the system, informing about the origin, production method, ingredients, safety, ethical and sustainability aspects during the process of bringing food from production to distribution on the table.
TITAN is a four-year project funded by the Horizon Europe. The overall aim of TITAN is to enhance food transparency in order to transform the food system into a demand-driven economy that provides consumers with healthy and sustainable food.
To achieve this, TITAN will demonstrate pre-identified technologies by deploying 15 pilots and new technologies – 8 pilots – that will be selected during the project with an open call. Innovations will involve the use of Blockchain, Internet of Things and Artificial Intelligence for enhance Healthy, Sustainable and Safe Food System.
It will also lay the foundations for an approach to meet future challenges, outlining a policy roadmap that puts transparency at the heart of the transforming food system.
-Pilot on microbiology of fermented food products, safety demonstration of food cultures:
Next-Generation sequencing technologies will be exploited for the rapid characterization of the microbial ecology in fermenting foods, the formation of multi-strain cultures applied to foods for fermentation and bio- preservation and the composition food supplements based on live microorganisms.
-Omics and molecular approaches for microbial and chemical quality of long shelf- life food products: Third-generation sequencing technologies will be exploited for microbial DNA will be exploited for microbial DNA to detect spore-forming pathogenic contaminants, antibiotic resistance genes and virulence factors. Results will be used for developing commercial PCR kits for detecting strains and/or genes of concern.

O5- 2

The need of a standard approach in microbiome science: the SUS-MIRRI.IT project

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The Microbial Resource Research Infrastructure (MIRRI) is the pan-European distributed Research Infrastructure for preservation, systematic investigation, provision and valorization of microbial resources and biodiversity. The SUS-MIRRI.IT project coordinated by the University of Torino and funded by Italy's National Recovery and Resilience Plan – PNRR, is granted by the European Commission's Next Generation EU program. Within the project, specific goals include: 1) define Standard Operation Procedures (SOPs) for the sampling of microbiomes from different sources studied, specifically fermented foods, plants, animals, humans, insects, soils and waters; 2) test performance study for the microbiome analysis 3) optimize the conditions and protocols for long-term preservation of microbiomes; 4) identification of analytical methods to follow the quality of microbiomes during storage. Samples collection and DNA extraction procedures are the backbone of the study of microbial community. The use of different protocols and procedures produces variability that can threaten the reliability, and the comparability in studying microbial communities. Therefore, to optimize data comparisons in the microbiome study, it is better to provide validated SOPs for every step. Our work was based on coordinating the standardization of protocols within the microbiome research programs of several matrices from the different ecosystems. Indeed, based on literature survey and integrating the expertise of the SUS-MIRRI.IT partners several SOPs were created, which were subsequent tested in intra-laboratory validation trails.

O5- 3

Innovative metabolomic and metagenomic approach applied to Parmigiano Reggiano PDO cheese to support traditional features

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Traditional fermented dairy products represent an invaluable resource for producing areas, also from an economical point of view, especially in the case of cheeses that are manufactured in rural/mountain areas. This is the case of Parmigiano Reggiano (PR) PDO cheese, produced by a variety of medium- to small-sizeddairies, located in a specific geographical area in Northern Italy. The bond between the cheese, manufactured with raw cow's milk and natural whey starter, and its area of origin, the so-called "terroir", is a key element that contributes to maintaining the high value of PR and makes all the supply chain sustainable in the area. At the same time, the organoleptic characteristics of PR cheese are intertwined with geographical origin since autochthonous populations of Lactic Acid Bacteria (LAB) are responsible for cheese fermentation, and together with technological processing parameters, characterise the final flavour of the cheese. In this study we performed a survey of 33 PR samples using shotgun metagenomics and metabolomic techniques, to identify associations between genome content of LAB and volatile compounds and organic acids production. Volatile compounds profile and organic acids content showed varying trends correlated with production seasons. Shotgun metagenomic data provided a depiction of the taxonomic composition of the microbial community, as well as of the encoded metabolic activities. As expected, metagenomes of PR were enriched with sequences for amino acids transport and utilisation, as well as proteolytic activities. An accurate description of the unique traits of PR cheese with innovative techniques that become available is essential for preserving and valorising the autochthonous microbial traits that are fundamental to maintaining the quality of cheese.

O5- 4

MinION sequencing of yeast mock communities to assess the effect of databases and ITS-LSU markers on the reliability of metabarcoding analysis

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Microbial communities play key roles both for humans and the environment. They are involved in ecosystem functions, maintaining their stability, and provide important services, such as carbon cycle and nitrogen cycle. Acting both as symbionts and as pathogens, description of the structure and composition of these communities is important. Metabarcoding uses ribosomal DNA (rDNA) (eukaryotic) or rRNA gene (prokaryotic) sequences for identification of species present in a site and measuring their abundance. This procedure requires several technical steps that could be source of bias producing a distorted view of the real community composition. In this work, we took advantage of an innovative "long-read" next-generation sequencing (NGS) technology (MinION) amplifying the DNA spanning from the internal transcribed spacer (ITS) to large subunit (LSU) that can be read simultaneously in this platform, providing more information than "short-read" systems. The experimental system consisted of six fungal mock communities composed ofspecies present at different relative abundances to mimic natural situations characterized by predominant and low-frequency species. The influence of the sequencing platform (MinION and Illumina MiSeq) and the effect of different reference databases and marker sequences on metagenomic identification of species were evaluated. The results showed that the ITS-based database provided more accurate species identification than LSU. Furthermore, a procedure based on a preliminary identification with standard reference databases followed by the production of custom databases, including only the best outputs of the first step, is proposed. This additional step improved the estimate of species proportion of the mock communities, especially for the sequences obtained with MinIon platform, and reduced the number of ghost species not really present in the simulated communities.

O5- 5

Yeasts against grape pathogenic fungi: a sustainable alternative toagrochemicals

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Biocontrol has become the promising approach in sustainable agriculture. This study explored the potential of epiphytic yeasts as natural antagonistic microorganisms for biocontrol. Yeasts isolated from Georgian *Vitis vinifera* (ssp. *sylvestris* and *vinifera*) were examined for their inhibitory activity against *Botrytis cinerea*, *Aspergillus carbonarius*, and *Penicillium expansum*.

In vivo trials on infected berries and *in vitro* tests using dual-culture plate and double petri dish tests allowed the selection of 19 yeasts as potential biocontrol agents. *Pichia kudriavzevii* UMY 1470, *Clavispora lusitaniae* UMY 1471, *Candida intermedia* UMY 189, *Cystofilobasidium infirmomiatum* UMY 205 and *Aureobasidium pullulans* UMY 201 showed the best antagonistic activity in dual-culture plate assays. In double Petri dish tests, 100 % inhibition was observed using *P. terricola* UMY 197, *P. kluyveri* UMY 210 and *Saccharomyces cerevisiae* strains against the three pathogens, and *P. kudriavzevii* UMY 1470 against

P. expansum and *B. cinerea*. Thus, the production of volatile organic compounds was verified by SPME/GC- mass spectrometry; *S. cerevisiae* UMY 1430 produced the most prominent inhibitory compounds (2- phenylethyl ester, oxalic acid, ethyl acetate, and heptanoic acid ethyl ester) against the three pathogens.

The yeasts' tolerance to copper, a commercial fungicide, and sulphur dioxide was tested. *C. intermedia* UMY 189, *A. pullulans* UMY 201, *Metschnikowia pulcherrima* UMY 1472, and *S. cerevisiae* UMY 1423 were the most resistant yeasts to copper while *P. kudriavzevii* UMY 1470 and *C. lusitaniae* UMY 1471 were tolerant to the fungicide (up to 1 g/L). However, all yeast strains were sensitive to an oenological dose of sulphur dioxide.

In conclusion, the inhibitory activity of the analysed yeasts proved to be promising; in field experiments will allow to fully examine their biocontrol potential.

O5- 6

Hanseniaspora valbyensis-bioprocessed pomegranate seeds to produce a novel food ingredient

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Pomegranate by-products are valuable sources of nutritionally important compounds. The aim of this study was to develop a large-scale consumption food, added with fermented pomegranate matrices, to be able to meet consumer's needs, in terms of nutrition and sustainability. To this aim, chemical composition, antioxidant activity, and microbiota of pomegranate matrices were analysed. *Hanseniaspora valbyensis* yeasts dominated (order of magnitude: 5 Log CFU/g) the matrices. Among four representative strains of *H. valbyensis* able to ferment pomegranate matrices, we selected S-L1, because it caused the highest increase in mineral content in seeds (e.g., ca. 162 mg of K/100 g of seeds fermented with S-L1 vs. ca. 104 mg of K/100 g of unfermented seeds). Then, we tried to design a *granola* snack supplemented with pomegranate seeds flour. In detail, we compared three theses of *granola*: G-FS, supplemented with fermented seeds flour; G-US, supplemented with unprocessed seeds flour; G-C, conventional granola (control). Compared to G-C, the use of pomegranate seeds flour increased antioxidant activity of the snack (ca. 60% DPPH· scavenging activity vs. ca. 40 % activity of G-C). The expected contents of calcium, iron, potassium, and zinc were higher in G-FS and G-C, compared to G-US, while lower energetic values are expected for the two fortified *granola* snacks. Fermentation of seeds with *H. valbyensis* S-L1 also contributed to significantly improve the overall acceptability of fortified granola snack (score on 1-9 scale: 6.3), making it more similar to the control (7.5) and more appreciated than granola containing unprocessed seeds (4.9). The results of this study could be of interest for food industries searching for novel foods, with added value in terms of nutritional quality and sustainability.

O5- 7

Exploiting of the agri-food waste and by-products potential to beused as substrate for bioplastic production through *Haloferax mediterranei* fermentation

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Polyhydroxyalkanoates (PHA) are biodegradable polymers, considered as alternative to petrochemical plastics. Among several microorganisms able to synthesize PHA, the halophilic *Haloferax mediterranei* has been largely investigated for its ability to produce the copolymer PHBV (belonging to PHA class) from organic waste using different carbon sources. The use of microorganisms and cheap substrates as agri-food waste represents a promising option for limiting environmental issues including waste disposal, costs for bioplastic production, and conventional plastic wastage thus promoting environmental sustainability. PHBVgranules are synthesized during cell growth under stress conditions and accumulated as energy storage materials. A downstream process follows the fermentative production of PHBV-rich biomass through cell biomass recover and bioplastic extraction and purification.

Haloferax mediterranei DSM 1411 was grown in a ricotta cheese exhausted whey (RCEW) fraction, previously enriched in lactose by a membrane-filtration system. Due to the *H. mediterranei* incapability to metabolize lactose, RCEW was subjected to β-galactosidase treatment to hydrolyse the disaccharide. To maintain the optimal osmotic conditions for *H. mediterranei*, the substrate was supplemented with NaCl and trace element solution (SL6). Under the experimental conditions an amount of PHBV of 1.18 g/L was obtained. A similar approach was employed to assess the suitability of a wasted bread-derived substrate. To reduce the costs for media supplementation, seawater was used instead of the SL6 and a water-based bioplastic extraction was set up. Under optimized conditions of fermentation, a bioplastic yield of 1.53 was obtained.

Currently, the use of waste and by-products from the potato supply chain as substrate for PHBV synthesys isunder investigation. Although *H. mediterranei* is able to directly metabolize starch, an amylase pre-treatmentled to an increase in PHB production (up to 0.500 g/L).

O5- 8

By-products fermentation: a step forward for the production of new antimicrobials

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Nowadays, food loss and waste are considered one of the major global issues. Indeed, the reduction of food loss and waste can be a way to lower production costs, improve food security and nutrition, and contribute to environmental sustainability, by releasing the pressure on natural resources. Globally, the food loss from post-harvest to distribution is estimated to be more than 14 % of the total produced. In terms of food groups, fruits and vegetables belong to the groups with a higher level of loss (more than 20 %). Moreover, during the food production chain, different types of by-products can be generated leading to the loss of nutrients and valuable compounds contained in food. So, by minimizing food loss and waste, and recovering by-products, most of the resources could be available to support the food system. In this context was born the patent "Production of antimicrobials from vegetable waste" (Italian patent n. 102019000006815 with international extension PCT/IB2020/054520). This project aims to produce antimicrobial extracts from the recovery of by-products obtained after the industrial processing of tomatoes as well as melons and carrots that were commonly left in the field due to quality standards (ripening stage, shape, etc.). Employing lactic acid bacteria, well recognized GRAS microorganisms, the by-products were fermented and the antimicrobial activity of the extract obtained was evaluated in vitro against the main foodborne/spoilage microorganisms as well as in model food matrices. The fermented extracts demonstrate to have antimicrobial activity and to be a promising way to be taken into account for food preservation. Starting from this work other by-products were involved in studies concerning the recovery of by-products through the fermentation process to better explore this interesting and innovative topic offering an option from waste disposal for the production of value-added products that can income-generating market opportunities.

Poster Session 1

FOOD microbiota as a tool for a sustainable future

P1

Comparative ethanol stress response and membrane fluidity in *Saccharomyces* and non-*Saccharomyces* yeast strains

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Yeast cells experience various stresses during the fermentation process, including ethanol-induced stress, which can alter cell membrane fluidity, compromise its integrity, and increase its permeability. This study aimed to investigate the effects of ethanol stress on the growth, membrane fluidity, and cell surface integrity of eleven yeast strains, including nine strains of *Saccharomyces cerevisiae* and two non-*Saccharomyces*. These strains are commercialized as wine starters by AEB SpA and a copy is preserved at the UnimoreMicrobial Culture Collection (UMCC).
The effect of ethanol on yeast strain growth was evaluated using selective media containing different ethanol concentrations, while the effect on membrane fluidity was assessed by measuring the generalized polarization of Laurdan (GP) using a spectrofluorometer. The results revealed a high ethanol tolerance of the
S. cerevisiae strains up to a concentration of 14 % (v/v), while non-*Saccharomyces* strains exhibited compromised growth when exposed to ethanol concentrations greater than 10 % (v/v). After 24 hours of exposure to 10 % ethanol, both *Saccharomyces* and non-*Saccharomyces* strains demonstrated a reduction in GP value and an increase in membrane fluidity. However, at higher ethanol concentrations (14 %), an increase in GP value was observed, suggesting a stiffening of the cell membrane. In the case of *S. cerevisiae* strains, this phenomenon could be explained by the need to counteract ethanol's effects, whereas for non- *Saccharomyces* strains, it could be related to a significant decline in cell viability.
This study highlights the distinct abilities of these strains to cope with ethanol stress in terms of growth and membrane fluidity. These results provide valuable insights for the development of resistant yeast strains thatcould lead to enhanced fermentation processes.

Part of this research was granted by AEB SpA and conducted as part of the project *"Investigation of FactorsAffecting Membrane Fluidity in Yeast Strains of Oenological Interest"* (Prot. 5662).

P2

Application of *Candida boidinii* and *Candida norvegica* to improvethe quality and safety of Nocellara del Belice table olives processed through Castelvetrano-style technology

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Table olives are daily consumed in Mediterranean countries and their production constitutes an economicallyimportant activity in this area. The methods mostly applied to process table olives in the major producing countries are Spanish- and Greek-style. However, in Sicily region, green olives are also consistently (31,000 tons/year) processed according to Castelvetrano-style consisting of a treatment with soda and salt addition, followed by storage in coldrooms (5-6 °C). Low temperatures are insufficient to limit the growth of undesired microorganisms (annual product losses of 10-15 %).

To this purpose, yeasts were isolated from brine samples during the last five olive campaigns in order to characterise phenotypically and genotipically interesting strains to be used to stabilise the fermentation process. Nineteen strains belonging to the species *Candida boidinii*, *Candida norvegica* and *Debaromyces hansenii* were further technologically screened. *C. boidinii* LC1 and *C. norvegica* OC10 showed the best results in terms of low temperature growth, pH and NaCl resistance were inoculated in experimental productions of Castelvetrano-style table olives. The process was monitored for physicochemical and microbiological parameters. At the end of the process (180 d), pH was around 5.0 and NaCl content was 8 %(w/v). The olives processed with the selected strains retained colour and pulp hardness was higher than that registered for the olives of the control trial. The dominance of inoculated strains (> 96 % over microbial community), strongly reduced the levels of potential spoilage and/or pathogen microorganisms. Sensory analysis showed an increase in colour, sweetness and crispness attributes for the olives of the trials inoculated with LC1 and OC10. Further studies are being prepared to validate the use of LC1 and OC10 strains under non-refrigerated conditions to increase the production sustainability.

The contribute was made with the co-financing of the European Union—FESR or FSE, PON Research andInnovation 2014-2020—DM 1062/2021.

P3

Addition of herbal supplement to *in vitro* fermentation of dairy cowrumen modulates the microbial community

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The diet of cows has a great impact on their ruminal environment altering the microbiota and metabolomics of the rumen. However, instead of changing the overall diet, feed can be supplemented with herbs to allow this modulation. We investigated the effect in vitro by fermenting herbs in rumen liquid from donor cows, analyzing the microbial community that developed, to evaluate the safety and possible benefits of the procedure.
Ruminal liquid was collected from three different dairy cows and mixed with media plus plant roots or oneof six different herbs. As controls we tested: a bottle with only media, a rumen bottle without any supplement and a bottle with supplementation of regular grass hay. Each mixture was fermented for 24 h.
Microbial DNA of rumen samples was extracted with commercial kit and sequenced by Illumina sequencing of V3 and V4 region of bacterial 16S rDNA. The raw reads have been analysed by QIIMNE pipeline and bacterial taxa identified by means of the greengenes 16S database. The alpha and beta diversities have been calculated by QIIME2 scripts.
The supplementation produced distinct microbiota significantly different ftom each other. Plant root supplementation caused the highest differences in rumen microbiota. Bacteriodales was the most abundant order, except for plant root supplemented fermentations, where Streptococcus was the most abundant genus. Different herbal supplementation can alter the microbial community of rumen in vitro. The *Streptococcus* abundance in plant root supplemented rumen samples could be a cause for concern, as the increase in *Streptococcus* genus in rumen microbiota could be related to a lowering of pH in rumen and cause acute acidosis to the dairy cows.

P4

Preliminary study on microbiome of industrial submerged vinegar

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Vinegar is a worldwide appreciated fermented product with numerous beneficial effects related to its daily consumption. Over the years, many studies focused on the detection of microorganisms involved vinegar production. In fact, for the selective and hostile nature of vinegar environment, bringing to light the complete microbiome composition of this matrix could have the purpose of isolating extremophile microorganisms and providing improvement strategies for food biotechnology. In this work, metataxonomic data analysis was carried out aiming to understand the vinegar microbiome changing during industrial submerged fermentation process. Samples were collected, DNA was extracted, and gene sequencing of V3- V4 region of 16S rRNA was performed. In detail, three collection points were chosen, i.e., bioreactor for the fermentation starter production, bioreactor after acetic fermentation, and bioreactor used to preserve product after whole fermentation process. According to operational taxonomic units (OTUs) analysis and taxonomic annotation results, the relative abundance of taxa for all samples was obtained. Moreover, alpha diversity for the analysis of microbial community complexity within each sample and beta diversity representing the differences between microbial communities among samples were realized. All findings indicated the presence of diverse microbial communities within samples, with predominant bacteria genus belonging to Acetobacter for all microbiome analysed. Moreover, the microbial composition was shown to slightly changed throughout fermentation process, underlining a high number of specific OTUs for the samples collected after acetic fermentation and after the whole fermentation process. This study improves the knowledge on bacterial population changes occurring during industrial vinegar fermentation and could be considered as preliminary study for a more in-depth research of vinegar microbiomes.

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P5

In *Streptococcus thermophilus*, urea hydrolysis paradoxically boosts acidification and reveals a new regulatory mechanism of glycolysis

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Streptococcus thermophilus is widely used in the dairy industry for the manufacturing of fermented milk andcheeses and probiotic formulations. *S. thermophilus* evolved from closely phylogenetically related pathogenic streptococci through loss-of-function events counterbalanced by the acquisition of relevant traits,such as lactose and urea utilization for the adaptation to the milk environment. The fate of ammonia and carbon dioxide derived by urea hydrolysis in several biosynthetic pathways have been depicted, and the positive effect of urease activity on *S. thermophilus* growth fitness and lactic acid fermentation in milk has been already addressed. This study aimed to assess the effect of urease activity on the growth and energy metabolism of *S. thermophilus* in milk. In milk, 13C-urea was completely hydrolyzed in the first 150 min of the growth, and urea hydrolysis was accompanied by an increase in cell density and a reduction in the generation time. By using energetically discharged cells with gene transcription and translation blocked, we showed that in the presence of fermentable carbon sources, urease activity, specifically the production of ammonia, could dramatically boost glycolysis and homolactic fermentation. Furthermore, we showed that ammonium ions were potent effectors of phosphofructokinase, a key glycolytic enzyme. Finding that ammonia-generating enzymes, such as urease, and exogenous ammonia act on phosphofructokinase activity shed new light on the regulatory mechanisms that govern glycolysis. Phosphofructokinase is the key enzyme known to exert a regulatory role on glycolytic flux and, therefore, ammonia as an effector of phosphofructokinase acts, in cascade, modulating the glycolytic pathway.

P6

In vitro faecal fermentation of *Tritordeum* breads and its effect on the human gut health

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Spontaneous fermentation of *Tritordeum* flour enhances the nutritional potential of this hybrid cereal. However, the effect of consumption of *Tritordeum* sourdough bread (SDB) on gut health remains to be elucidated. This study investigated the effect of *in vitro* digestion and faecal fermentation of SDB compared to that of traditional baker's yeast (BYB) *Tritordeum* bread. After 24-h anaerobic faecal fermentation, both SDB and BYB (1% w/v) induced an increase in the relative abundances of *Bifidobacterium, Megasphaera, Mitsuokella*, and *Phascolarctobacterium* genera compared to baseline, while concentrations of acetate and butyrate were significantly higher at 24 h for SDB compared to those for BYB. Integrity of intestinal epithelium, as assessed through *in vitro* trans-epithelial electrical resistance (TEER) assay, was slightly increased after incubation with SDB fermentation supernatants, but not after incubation with BYB fermentation supernatants. The SDB stimulated *in vitro* mucosal immune response by inducing early secretion of inflammatory cytokines, IL-6 and TNF-α, followed by downregulation of the inflammatory trigger through induction of anti-inflammatory IL-10 expression. Overall, our findings suggest that Tritordeum sourdough can modulate gut microbiota fermentation activity and positively impact the gut health.

P7

Lactic Acid Bacteria fermentation for protein functionalization: thecontext of climate smart food Innovation using Plant and Seaweed proteins from Upcycled Sources (IPSUS) project

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Food choices impact human and planetary health. The negative environmental impact of the food system, increasing food insecurity and the prevalence of unhealthy diets, are driving policymakers, scientists, companies, and consumers to demand sustainable solutions. In particular, the transition to more sustainable sources of proteins is becoming crucial. Plant-based proteins are currently the fastest growing food trend, butare unsustainably dependent on soy. The IPSUS project will exploit interdisciplinary and eco-innovative approaches to upcycle plant and seaweed proteins from agri-food raw materials. The quantity, quality, and upcycling opportunities of six protein-rich food loss and waste (FLWs) (pumpkin, hazelnut, grape, potato, brewers' spent grain, seaweeds) across the value chains will be investigated in UK, Italy, Romania, Turkey, and Morocco to address Net Zero opportunity by linking sustainable protein shift and food waste valorization.

In the last years, many studies focused on the improvement of the biological value, functionality and palatability of plant-based proteins. The fermentation process can be considered as a valid strategy to enhance their technological, nutritional, and organoleptic properties by obtaining high-value protein extracts.Lactic Acid Bacteria (LAB) are known to produce high-value molecules, such as lactic acid, aroma compounds, and a variety of bioactive compounds with potential health benefits.

Within the IPSUS project, LAB strains will be selected among those belonging to the University of Parma culture collection (UPCC), according to their fermentative ability and their different phenotypic characteristics. Their growth capacity, and proteolytic activity, together with their ability to produce exopolysaccharides (EPS) and lactic acid will be evaluated.

In the context of the IPSUS project, LAB fermented plant based proteins will be used to develop innovativeplant-based meat and cheese alternatives.

P8

Companilactobacillus alimentarius isolated from spontaneously fermented sausages: safety and technological features

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Companilactobacillus alimentarius can be found in traditional fermented foods, depending on origin area. This species is particularly relevant in Spanish fermented sausages, with isolation frequencies similar to *Latilactobacillus sakei* and *Latilactobacillus curvatus*, usually dominant in fermented meat products. Moreover, *Comp. alimentarius*, recognised as QPS (Qualified Presumption of Safety) by EFSA, can play an important role in fermentative or ripening processes. Despite this, they are not used as selected starter cultureand the knowledge about their safety and technological features is scarce in literature. For these reasons, 14 *Comp. alimentarius* strains, isolated from spontaneously fermented sausages collected in Andalusia region (Spain), were characterised regarding their safety aspect (amino biogenic potential and antibiotic resistances (AMR)) and technological properties. Regarding safety issues, 64.5 % of the tested strains produced tyramine, even if in different amounts (from 15.1 to 1156.6 mg/L). Histidine decarboxylase activity was found in 28.6 % of the strains, among which two were able to accumulate concentrations higher than 600 mg/l in decarboxylase medium. AMR analysis showed that 57 % of *Comp. alimentarius* strains presented at least one resistance, with 50 % of them having multi-resistances. Finally, the selected strains were studied regarding their growth performances in the presence of different salt concentrations (0 %, 2.5 % and 5 %) and at different incubation temperatures (10 °C, 20 °C and 30 °C), monitoring OD at 600 nm and evolution of pH. The data were modelled with Gompertz equation to obtain predicted growth parameters and the results showed a significant variability among the strains, evidencing a high phenotypic diversity. Strains considered safe will be tested as potential starter cultures in fermented sausages, also considering their effect on the microbiota and on the aroma profile of ripened fermented sausages.

P9

Diving into the depths of Atlantis II Deep: unravelling microbiomeadaptation in the Red Sea's hottest brine pool

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The Atlantis II Deep in the Red Sea is a deep hypersaline anoxic basin (DHAB) known for its polyextreme conditions. It is characterized by high temperatures reaching up to 69 °C, high salinity (up to 6 times that of regular seawater), high pressure (depth of 2190 m), low pH (5.0), oxygen depletion, and the input of metal-rich hydrothermal fluids from oceanic crust. The hydrothermal activity determinesthree vertical stratified convective layers. Despite the harsh conditions, this DHAB supports a diverse microbial community that varies across the differentcompartments along the vertical profile. The presence of multiple convective layers, each exposed to progressively increasing temperature and salinity stress, provides an opportunity to study the variations occurring in the microbial community and the metabolic strategies adopted by specialized microorganisms to cope with thermal stress under anoxic hypersaline conditions. In our study, we reconstructed 333 metagenomic-assembled prokaryotic genomes (MAGs) from 15 fractions spanning from the overlaying seawater to the three brine bodies and their respective transition zones. Through this analysis, we observed a progressive increase in archaeal abundance toward the hottest and saltiest brine body (69 °C) compared to the bacterial-dominated community associated with the brine-seawater interface. Among the MAGs assembled in the most extreme layer, we detected the presence of metabolic traitswhich are involved in extending microbial thermal tolerance. Studying the microbial community of Atlantis II Deep expands our understanding of microbial diversity in DHABs and provides opportunities to investigate the ecological roles and evolutionary adaptations of endemic microorganisms that are selected by extreme environmental conditions challenging the boundaries of life.

P10

Comprehensive assessment of bacterial community viability in natural whey starter cultures by flow cytometric approach

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The natural whey starter consists of a lactic microbial community perpetuated daily by fermenting the whey from the previous day's processing. These natural cultures play a crucial role in the acidification process of the cheese mass, which must lead to the complete breakdown of lactose in the first few hours after its production. The lactic acid bacteria in the whey starter act first, through their metabolism, as living cells and then as released enzymes, participating in the complex biochemical phenomena that occur during cheese ripening. The viability of the cells plays a key role in the acidifying activity required during the first hours of curd acidification, in parallel to the microbial composition. Analytical methods based on plate counts have long been used to evaluate the number of cultured cells. The disadvantage of these methods is that they have high variability and take days to obtain results. In this work, we focused on evaluating the physiological state of the lactic bacterial cell community of natural whey starter samples. This work involved a comparisonof bacterial enumeration by classical methods using whey agar medium, MRS, and M17, and flow cytometry on natural whey samples. The flow cytometry results were in good agreement with a tendency towards overestimation. Flow cytometry has also introduced other parameters to assess natural whey by measuring cell physiological status. In addition, two fluorescent dyes used in flow cytometry resulted in the assessment of cell wall damage and metabolic activity and therefore enabled the estimation of the number of cells even under sub-optimal physiological states. In conclusion, we discovered that determining the physiological condition of the cells served as a potential indicator for predicting its acidifying activity.

P11

How PVC microplastics affect soil chemical and microbiological parameters

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One of the most urgent global environmental concerns is the presence of microplastic pollution in various ecosystems, including soil. Among them, the presence of polyvinyl chloride (PVC) is particularly problematic due to its resistance to degradation, which led to a long persistence in soil ecosystem.
A one-year microcosm experiment was set up to test the impact of PVC (0.021 % w/w, corresponding to an average annual amount of plastic polluted compost from 7 to 35 t ha-1) on soil physical/chemical parameters, namely CO_2 emissions, fluorescein diacetate (FDA) activity, total organic C (TOC), total N, water extractable organic C (WEOC), water extractable N (WEN) and SUVA254. Moreover, the impact of PVC on the structure of soil microbial communities was analyzed at different taxonomic levels targeting the V3-V4 region of bacterial 16S gene and the fungal ITS2 rDNA region by NGS sequencing (Illumina MiSeq).
Although some fluctuations were found, some significant trends were observed in both chemical and microbiological parameters. Significant ($p<0.05$) variations of CO_2 emission, FDA activity, TOC, WEOC and WEN were found in PVC-treated soils at different incubation times. Furthermore, the presence of PVC particles in soil determined a significant ($p<0.05$) taxon-dependent variation of the abundances of specific bacterial and fungal taxa: Candidatus_Saccharibacteria, Proteobacteria, Actinobacteria, Acidobacteria and Bacteroides among bacteria, and Basidiomycota, Mortierellomycota and Ascomycota among fungi. A significant ($p<0.05$) reduction of both number and dimensions of PVC particles was found after one year of incubation, suggesting a potential contribution of microorganisms in degrading the polymer.

P12

A *core* microbiota as a microbial heritage of Pecorino Toscano PDO cheese

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National dairy sector is composed of a multitude of PDO cheeses, and their defense must necessarily foreseea deep characterization. Pecorino Toscano is a PDO Italian cheese characterized by a close association between cheese and production area. In this regard, the defense of this microbial heritage is to be considered of great importance to preserve and enhance its qualitative attributes. Environmental fluctuations keep the microbial community in continuous change, and in this dynamic scenario a constant review and implementation of the existing microbial collections is required, to better define and select autochthonous technological strains. For these reasons, this study aims to define a core microbiota, composed of autochthonous "key" microorganisms, useful for the production process and selected for their safety profile and highly technological properties. Seventy-three isolates were collected from fresh and ripened cheeses, identified by 16S RNA gene sequencing, genotyped by Rep-PCR analysis, and compared with other strains already present in the collection, for a total of 115 isolates. For all the samples, the antimicrobial resistance patterns were evaluated by MIC analysis, demonstrating that strains belonging to non-starter Lactic Acid Bacteria (NSLAB) act as *reservoirs* for antibiotic-resistances to a greater extent than starter Lactic Acid Bacteria (SLAB) species. The potential acidifying ability was investigated by impedance analysis, identifying two distinct clusters, characterized by diverse adaptation abilities and acidifying performances.
Lastly, HS-SPME-GC/MS method was applied for the investigation of the volatile profiles' dynamics in cheese and strains inoculated in ewe's milk, revealing 50 volatile compounds, 22 of which were also detected in cheese. In the light of the analyses performed, it was possible to establish a core microbial collection, composed of 29 biotypes, which includes the most technologically important strains characterizedby a safety profile, both relevant aspects to save and enhance a microbial collection of a production process.

P13

Does climate change influence the milk quality of Parmigiano-Reggiano production? A focus on years 2020-2022

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Climate change is causing global warming, which is a major concern affecting the agri-food industry. In particular, livestock sector must deal not only with the direct heat stress on animals, but also changing in soilfertility, water availability, crop yield, and pathogen circulation. Cattle welfare and milk characteristics are strongly affected by heat stress. Although weather influence on dairy cattle milk production has been broadly investigated concerning seasonal variations, a lower number of studies analysed this phenomenon over multiple years.

In this study, we analysed over three years the characteristics of milk used for Parmigiano Reggiano (PR) production, a raw-milk, cooked, long-ripened cheese considered one of the most famous Italian Protected Designation of Origin products. More than 15,000 raw milk samples coming from a total of 244 dairy farms representative of the whole PR production area (lowland, hill and mountain territories) were analysed for thequality parameters commonly assessed for milk payment, i.e. fat and casein contents, coagulation properties and microbiological profile. The results have been correlated with the daily weather conditions of the specific farm location.

Our results confirm seasonal trends already reported in literature. During the warm season, reduction of titratable acidity and worsening of milk coagulation properties have been observed, making the milk less suitable for PR production. A decrease in fat and protein contents during summer have been also observed, while total bacterial count increased. Lactose content and spore counts were found to be not correlated withtemperature changes. Furthermore, intriguing variations were correlated with the temperature increase registered from 2020 to 2023 and the farm location.

Since raw milk quality strongly affects PR final characteristics, the monitoring of milk variations in functionof the climate change is essential to predict the PR compliance to the disciplinary standards and its economicsustainability.

P14

Lacticaseibacillus sp. strains isolated from raw milk: screening strategy for their qualification as adjunct culture in cheesemaking

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Microbiological and biochemical changes of the curd are crucial factors to produce raw-milk, long-ripened cheeses. The microbial ecology fundamentals of this cheese variety consist of a complex interaction between starter Lactic Acid Bacteria (LAB) and non-starter LAB (NSLAB). The need to standardize cheesemaking and accelerate ripening has driven the use of several NSLAB as adjuncts secondary starters. From this perspective, this work focused on the isolation and characterization of NSLAB from raw cow's milk from 20 dairies producing Grana Padano, the PDO cheese most exported in the world. Although the aromatic properties of NSLAB are paramount, other phenotypic traits need to be considered, such as the capability to endure the technological parameters encountered during cheese making. Using the strategy of low- temperature spontaneous fermentation, 122 strains have been isolated; morphology and genotypic characterization were applied to select the 10 most diverse strains belonging to *Lacticaseibacillus* sp. for subsequent phenotypic screening. Carbon and nitrogen source utilization and sensitivity to different chemicals were tested by phenotypic microarray. The impedance technique was used to evaluate the growth performance in milk in combination with the application of thermal stress, salt, and lysozyme. Finally, the production of volatile compounds after strains' growth in milk was assessed, using solid-phase micro- extraction coupled with gas chromatography-mass spectrometry technique. The phenotypic characterization has been discussed to reach the elements necessary to qualify the best adjunct strains to produce raw-milk, long-ripened cheeses. The two best strains were 5959 *Lb. paracasei* and 5296 *Lb. paracasei*, even if their antibiotic resistance must be correctly measured before their employment. Other strains with interesting aromatic capabilities but lower heat resistance were 5293 *Lb. paracasei*, 5649 *Lb. paracasei* and 5780 *Lb. paracasei*, which could be good candidates as adjunct strains for uncooked-cheese production.
Intriguingly, strains 5993 *Lb. paracasei* and 5293 *Lb. paracasei* showed metabolic capabilities interesting for the fermentation of plant-based food matrices, food by-product and imitation cheeses for vegans.

P15

Development of a droplet digital PCR assay for a fast and sensitive detection and quantification of aerobic spore formers in plant- based food supplements

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TITAN is a four-year project funded by the Horizon Europe and dedicated to the development of analytical and digital solutions for the transparency of the food supply chain, to promote food traceability, safety, and health. To achieve this object, TITAN will build innovative, rapid, and effective solutions, based on omics and molecular approaches, which can be applied even by companies, with the final aim to support them in the management of quality system controls, strengthening consumer trust.

A particular attention is given to the new and expanding food categories on the market: our pilot will focus on the safety of long shelf-life plant-based products, whose consumption among the population registered a sharp increase during the last decade, due to ethnical reasons, allergy and intolerance problems and to consumer preferences to choose dairy substitutes.

Although outbreaks of microbial contamination of this food chain have already been reported, in-depth microbiological studies regarding these categories of products still lack and, according to the recalls happened worldwide, it can be confirmed that spore formers are the most feared agents of contamination.

For all the reasons mentioned above, the first method we developed is a droplet digital PCR (ddPCR) assay for a rapid and accurate detection and quantification of aerobic spore formers in plant-based food supplements (in particular, for *B. cereus*, *B. subtilis* and *B. licheniformis*). The developed assay was tested on a considerable number of products (tablets, capsules, opercula, and liquids) which confirmed the highly sensitiveness and accuracy of this methodology. Based on our results, it was also demonstrated as the ddPCR methodology represents a very rapid and promising tool for evaluating the microbial load in food. To our knowledge, this is the first ddPCR assay developed for the detection and quantification of aerobic spore formers in plant-based products.

P16

Antagonistic activity against foodborne and human pathogens, biofilm formation, and antiadhesion activity of lactobacilli from different source

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Antagonistic activity against pathogens as well as ability to form biofilm are considered key properties of probiotic strains. The formation of biofilm, along with allowing organisms to persist under stressful conditions, by promoting mucosal colonization, can interferes with the growth and adhesion of foodborne and human pathogens. The present study aimed to test sixty lactobacilli strains, isolated from both food and human ecosystems, for the abilities to antagonize pathogens, produce biofilm, and exert antiadhesion activity against *Candida* species. Cells and cell free supernatant (CFS) of lactobacilli were tested for antagonistic activity against 16 pathogens (*E. coli*, *L. monocytogenes*, *S. aureus*, *S. enterica*, *C. jejuni*, *Candida* sp.) by agar disk-diffusion assay. Based on the diameter of the inhibition zone, the antagonistic activity was ranked as absent, low, intermediate, and high. The ability to produce biofilm was evaluated on 96-well polystyrene microplates by measuring the optical density at 595 nm. The strains were scored as from no biofilm producers to very strong biofilm producers. The antiadhesion activity of the lactobacilli strains against pathogens was evaluated by precoating and coincubation experiments. Overall, a broad spectrum of antagonistic activity was shown by all lactobacilli. Based on the CFS treatments, the antagonistic activity was attributed to organic acid. All food-derived lactobacilli showed strong or very strong ability to produce biofilm whereas high variability was revealed among human-derived lactobacilli. Most of the tested strains showed antiadhesion activity in both precoating and coincubation assays. In particular, food-derived lactobacilli exhibited promising antiadhesion abilities against *Candida krusei* DSM 70079 in the co- incubation assay, whereas human-derived strains antagonized the adhesion of *Candida albicans* ATCC 10231 and *Candida tropicalis* DSM 5991 in pre-coating and co-incubation assay, respectively.

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P17

Screening of bacterial strains from spontaneously Mediterranean fermented sausages for their potential use as bio-protective and starter cultures in fresh processed meat

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Traditional naturally fermented products of Mediterranean origin can be considered a reservoir of biodiversity that can be exploited in industrial processes with the aim of ensuring food safety and consumer satisfaction. Nowadays, the market demands products with a clean label that do not contain synthetic preservatives, and bio-protective cultures can be seen as a possible solution. The scope of this study is to apply indigenous protective cultures to provide meat products of high quality and safety, minimizing food waste. A total of 151 biotypes of Lactic Acid Bacteria (LAB) were isolated from 15 naturally fermented sausages of Mediterranean origin and tested for safety traits (absence of antibiotic resistance genes and biogenic amine production), inhibition capacity against foodborne pathogens and bacteriocin production. The best 5 strains, belonging to the species *Latilactobacillus sakei* (2M-7, ZK-39), *Latilactobacillus curvatus* (KN-55), *Pediococcus acidilactici* (PE) and *Lactiplantibacillus paraplantarum* (PL), were used for a shelf- life test and sensory analysis in fresh sausages stored under conditions of thermal abuse (6°C). Counts for LAB, Enterobacteriaceae, *Brochothrix thermosphacta* and Staphylococci were carried out at 5 times of analysis (0, 2, 6, 9, 12 days). Sensory analysis was used to evaluate the visual and olfactory characteristics ofthe treated sausages. The results showed that 4 strains had a bioprotective action (reduction of 1-2 log CFU/g) against species of the Enterobacteriaceae family. Instead, no improvement was observed against *B. thermosphacta*. The same samples presented an increase in firmness and chewiness when Texture Profile Analysis was applied. Sensory analysis showed that the strain 2M-7 resulted in an improvement of the olfactory characteristics. Our work highlights the relevance of the use of autochthonous bacteria to enhance sustainability of traditional food productions.

P18

Herbal and local fruit cultivars may improvethe sustainability of craft specialty beers

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By adding unique flavors and bioactive compounds, as well as by acting as a reservoir for microbial starter strains, local herbs and fruit cultivars can significantly improve the quality of craft beers. Here, the bacterial and fungal populations of the Bisucciu fruit, a Sardinian apricot variety, were isolated and characterized. Moreover, the phytochemical composition, the antimicrobial, antioxidant, and aromatic properties of leave extracts of Sardinian spontaneous shrub species (*Mirtus communis* L., *Pistacia lentiscus* L., and *Artemisia arborescens* L.) were analyzed. In particular, the antimicrobial activity of the extracts was evaluated on beer spoilage LABs (*Levilactobacillus brevis*, *Fructilactobacillus lindneri*, *Lactiplantibacillus plantarum*, *Lacticaseibacillus casei*, *Leuconostoc citreum*) and common food pathogens.
From the 16S rDNA and ITS sequence analyses of 68 epiphytic isolates, 5 bacterial species and 19 fungal species were identified, being *Aureobasidium pullulans* and *Rhodotorula glutinis* the dominant fungal species. *Enterococcus mundtii* and *Frigoribacterium faeni* were the most represented species among bacterial isolates. Fermentation trials in beer wort and beer wort supplemented with Bisucciu apricot puree, allowed selecting 4 yeast strains belonging to *Pichia kudriavzevii*, *Hanseniaspora uvarum*, *H. pseudoguilliermondii*, and *H. clermontiae* as potential brewing starters in mixed fermentations. Indeed, these strains were able to produce from 0.57 % to 0.74 % (vol/vol) of ethanol, and to influence the sensory qualities of the beer by raising the ester and alcohol fractions while decreasing terpenes. Leaves extracts from *M. communis* L. showed antimicrobial activity on all microorganisms tested, except for the hop resistant *Levilactobacillus brevis*. The analysis of the polyphenolic composition of the *M. communis* L. extract highlighted the presence of flavonoids (conjugates of myricetin and vitexin), hydrolysable tannins (gallomyrtucommulone, pedunculagin and punicalin) and phenolic acids (quinonic acid isomers).
Results obtained highlighted the importance of the proper exploitation of plant and microbial biodiversity toimprove the quality and sustainability of fermented foods.

P19

The wooden shelf surface and cheese rind exchange microbiotareciprocally during ripening

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The rind acts as a protective barrier for internally-bacterial ripened cheeses. Unlike surface-inoculated smear cheeses, centripetal maturation is not assumed to occur in these cheeses. This research was aimed to evaluate the microbial diversity of the wooden shelves used for the ripening of Protected Denomination of Origin (PDO) Pecorino di Filiano and Protected Geographical Indication (PGI) Canestrato di Moliterno cheeses. The microorganisms associated with the rind of these cheeses were also investigated. Both wooden shelf surfaces and cheese rinds were sampled by brushing method to collect their biofilms. Wooden shelves showed levels of total mesophilic microorganisms (TMM) between 5.6 and 7.2 Log CFU/cm^2, while cheese rinds between 6.1 and 8.8 Log CFU/cm^2. The major dairy pathogens were never detected, while mesophilic and thermophilic coccus Lactic Acid Bacteria (LAB) dominated the surfaces of all wooden shelves and cheese rinds. LAB community was dominated by *Enterococcus* sp., *Leuconostoc* sp., and *Marinilactibacillus* sp. Among yeasts, *Debaryomyces* sp., *Candida* sp., and *Talaromyces* sp. were identified, while *Aspergillus* sp., and*Penicillium* sp., dominated the community of filamentous fungi. MiSeq Illumina analysis identified 15 phyla, 13 classes, 28 orders, 54 families, and 56 genera. *Staphylococcus* sp. was identified from all wooden surfaces, with a maximum abundance of 71 %. *Brevibacterium*, *Corynebacterium* and halophilic bacteria were detected in almost all samples. This study confirmed that the wooden shelves used for cheese ripening are microbiologically active and represent safe systems. Furthermore, the results of this work clarified the transfer flow between wooden shelves and PDO Pecorino di Filiano and PGI Canestrato di Moliterno cheese surfaces: wooden shelves mainly transferred smear-active bacteria to cheese rind, contributing to the development of the final organoleptic characteristics, while cheeses transferred LAB defining for the safety aspects of the shelves.

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P20

Phycoremediation: an indigenous microalga from a constructedwetland as a strategy for urban wastewater

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A sustainable and promising solution for wastewater treatment is represented by microalgae. This study was aimed to compare the efficacy of an autochthonous microalgae species with those of *Chlorella vulgaris* and *Scenedesmus quadricauda*, in urban wastewaters treatment. In detail, microalgae isolates were obtained from a free water surface pond in a wetland system. Water samples from the Imhoff tank of the same wetland system, at two different times (MW1 and MW2), were obtained and treated in 2 L Erlenmeyer flask at laboratory scale at 25 ± 2 °C, with a photoperiod 16:8 (day:night), a light intensity of 100 µmol photons·m-2 s-1, supplied with air. The water samples were singularly inoculated with: *C. vulgaris* ACUF110; *S. quadricauda* ACUF581; an autochthonous microalga, identified as *Klebsormidium* sp. K39, at a final concentration of 1.6, 2.2 and $1.8*10^9$ cells/L, respectively. Their removal efficacy was monitored for chemical and microbiological parameters as Total Kjeldahl Nitrogen: TKN; Total Phosphorous: TP; Biochemical Oxygen Demand: BOD5; Chemical Oxygen Demand: COD) *Escherichia coli*.
Results demonstrated that indigenous microalga is comparable to *S. quadricauda* and *C. vulgaris* in terms of removal efficacy for TKN, BOD5 and COD in wastewater samples. In particular, *Klebsormidium* sp. K39 exhibited a higher TP removal rate in MW1 compared to the other two species. Moreover, after 15 days, *E. coli*, starting from an initial density of 6.8 Log CFU/mL, was never detected in any microalgae treatments, accomplishing the EU Regulation 2020/741. In conclusion results revealed that the autochthonous *Klebsormidium* sp. K39 strain is a promising strategy for wastewater treatment.

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P21

Investigating differences in microbial communities associated tograpevines cv. Barbera grafted on distinct *Vitis* rootstocks

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Many are the factors driving microbial diversity in vineyards. In particular, plant material chosen at vineyardestablishment could significantly affect microbial populations colonizing below and above-ground plant organs. In viticulture, *Vitis vinifera* cultivars are compulsorily grafted on *Vitis* sp. rootstocks to promote resistance to phylloxera.
The aim of this work is to characterize the microbial diversity of bacteria and fungi associated with leaves and rhizosphere of *Vitis* sp. according to the use of different *Vitis* rootstocks. The study was conducted in the Colli Piacentini wine region, in a vineyard planted in 2016, where, in different rows, vines cv. Barbera were grafted on three *Vitis berlandieri* x *Vitis riparia* rootstock genotypes (Kober 5BB, 420A and SO4), one *Vitis berlandieri* x *Vitis rupestris* selection (1103 Paulsen), and two recently bred complex *Vitis* complex hybrid rootstocks (M2 and M4). The six grafting combinations (Barbera x: Kober5BB, 420A, SO4, 1103 Paulsen, M2, M4) represent the treatments of the present work. In 2023, samples of leaves and rhizosphere will be collected at two key phonological stages: flowering and veraison. Concomitantly, midday leaf water potential will be determined in order to assess plant water status for any of the grafting combinations.
Weather data and seasonal soil moisture and temperature will be collected by a weather station located in thevineyard and equipped with soil probes. On the collected samples, bacterial and fungal populations will be studied via culture-independent technique based on Next-Generation Sequencing (NGS). Results will clarify the impact of the *Vitis* rootstock genetic background on microbial communities populating vineyard below- and above-ground layers, and how the populations vary according to differentwater resource availability and will contribute to the study of the eventual rootstock x microorganisms' interactions in the sight of a more resilient viticulture.

P22

The genus *Microbacterium*: a bacterial community resistant to sanitisation processes in long-life milk

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Microfiltration and pasteurization are treatments carried out to guarantee the safety of milk and to elongate the shelf life. These sanitization processes strongly modify the composition of the microbial population in raw milk. Microfiltered milk samples produced over three months and in two different industrial dairy plants were studied. The microbial analysis allowed us to identify *Microbacterium* sp. as dominant and other spoiling bacteria belonging to *Bacillus*, *Acinetobacter*, *Micrococcus*, *Staphylococcus*, *Pantoea*, and *Escherichia*. Microorganisms were identified after colony isolation and by 16S rRNA sequencing. To accurately identify *Microbacterium* species, gyrB gene similarity analysis was performed, and *Microbacterium lacticum* and *Microbacterium paulum* resulted being the two dominant species. The role played by *Microbacterium* sp. in milk is unknown, and a lab-scale experiment was carried out to evaluate the effects. Most *Microbacterium* isolated strains can grow in milk with moderate acidification activity. The strains were exposed to pasteurization treatment, and the isolated strains survived when a combination of time and temperature similar to industrial conditions was used. Antibiotic resistance was evaluated in the isolated *Microbacterium* strains, and resistance to clindamycin and ciprofloxacin was found.

P23

Identification and selection of prospective probiotics for enhancing gastrointestinal digestion: application in pharmaceutical preparations and dietary supplements

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Our study investigated the effectiveness of 446 strains of Lactic Acid Bacteria (LAB) belonging to different species and isolated from diverse sources (food, human, and animal) as potential probiotic candidates, withthe perspective of producing dietary supplements or pharmacological formulations suitable for enhancing gastrointestinal digestion. The survival capability of all the isolates under harsh gastrointestinal tract conditions was evaluated, in which only 44 strains, named high-resistant, were selected for further food digestibility investigations. All 44 strains hydrolyzed raffinose and exhibited amino and iminopeptidase activities but at various extents, confirming species- and strain-specificity. After partial in vitro digestion mimicking oral and gastric digestive phases, food matrices were incubated with single strains for 24 h.
Fermented partially digested matrices provided additional functional properties for some investigated strains by releasing peptides and increasing the release of highly bio-accessible free phenolic compounds. A scoring procedure was proposed as an effective tool to reduce data complexity and quantitively characterize the probiotic potential of each LAB strain, which could be more useful in the selection procedure of powerful probiotics.

P24

Ecological variability of raw cow milk and its impact on microbial and metabolic changes during Caciocavallo cheese ripening

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Raw cow milk is one of the most complex and unpredictable food matrices shaped by the interaction between biotic and abiotic factors. Changes in dairy farming conditions impact the quality and safety of milk, which largely depend on seasonality. Changes in microbiome composition and relative metabolic pathways are derived from microbial interactions, as well as from seasonality, mammary, and extramammary conditions. Breeding data from >600 Apulian farms were examined, and the associated physicochemical parameters were processed by a reductionist approach to obtain a sample subset. We investigated the microbiological variability in cultivable and 16S rRNA sequencing microbiota as affected by seasonal fluctuations at two time points (winter and summer seasons). We identified families (Xanthomonadaceae, Enterobacteriaceae, and Pseudomonadaceae) whose increased abundance during winter may cause a shift toward a pathobiont microbial niche that leads to lower milk quality. Apulian summer season conditions were advantageous to the presence of specific taxa, i.e., Streptococcaceae (i.e., *Lactococcus*) and *Limosilactobacillus fermentum*, which in turn may favor better milk preservation. Selected milks from summer season were used to produce Caciocavallo cheeses in four different Apulian dairy industries following the same production process. Dynamic changes in cheese microbiota composition and VOCs profile were investigated. Based on metataxonomics , bulks milks significantly differed at genus level, while the used natural whey starters (NWSs) mainly differed for the presence of *Lactobacillus* or *Streptococcus* as dominant taxon. Deeply, the NWSs clustering relying on qPCR results exhibited that the diversity existing between NWSs was based on the detection of few LAB species. Volatile profile of cheeses after 30 and 60 days of ripening was characterized by more than fifty VOCs. Noteworthy, based on the analysis of variance, the microbiota β-diversity emphasized the significant contribution of the dairy companies in shape the Caciocavallo cheese microbiota under a dependent way during ripening.

P25

Agri-food by-products and lactic acid bacteria as a strategy to improve the quality and functionality of "Primo Sale" cheese

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In Europe alone, 22.4 million tonnes of food by-products (BPs) are generated each year. However, they still contain valuable compounds, which can be valorised by the food industry. For this reason, some BPs have been selected to be used as ingredients for the production of "Primo Sale" cheese. Orange peels (*Citrus*) and *Equisetum arvense* (Eq), a weed plant, were chemically and functionally characterised and used for the formulation (2 % and 1 % w/w, respectively) of "Primo Sale" cheese. Three functional Lactic Acid Bacteria (LAB) were also used in addition to the starters. The cheese samples were monitored over 29 days at 4 °C for the indigenous microbiota, starters' viability, volatile compounds and antioxidant activity. Results showed that the addition of the BPs did not affect the starters' viability, which was higher than 8 Log units, while growth of spoilage microbiota was inhibited. Inhibition of *Pseudomonas* sp. was observed in the cheeses added with the LAB. Moreover, *Citrus* and Eq had a synergic effect resulting in 2 Log units lower than the control with LAB. Antioxidant activity of the cheeses increased with the addition of both BPs compared to the controls, with the best results observed for the *Citrus*-added cheeses. Furthermore, all the matrices showed a significant increase of the antioxidant activity over time ($p<0.05$), doubling at the end of storage. The analysis of volatile compounds showed that the content of the organic acids increased overtime for the samples with the presence of residues, and particularly for short-chain fatty acids. In conclusion, the addition of BPs to the cheeses positively impacted the shelf-life without affecting the growth of the LAB strains. In addition, they improved certain properties such as the antioxidant one and allowed differentiation in the volatile profile. Finally, the use of these substances can increase the prebiotic effect and fibre content.

P26

Torulaspora delbrueckii in sequential fermentation with improvednative *Saccharomyces cerevisiae*: bio-protectant and aroma profileenhancer in wines with reduced sulfites

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In winemaking the use of selected cultures is a suitable strategy to control the fermentation process and improve organoleptic profiles and specific aroma compounds for the production of distinctive. Among the different non-*Saccharomyces* wine yeasts used in mixed fermentation with *S. cerevisiae* in winemaking, *Torulaspora delbrueckii* showed several features that positively affect the wine quality. In this study it was evaluated the use of a selected strain of *T. delbrueckii* in sequential fermentation with native S. cerevisiae strains already selected and tested for low solphite wine production. The final goals would be the evaluation of potential biocontrol and aroma-enhancing action of *T. delbrueckii*, in organic wines. Results obtained seems to confirm the supposed antimicrobial activity of *T. delbrueckii* that was able to control indigenous yeasts. Although the effect of the sulfur dioxide produced a complete reduction of these within the first two days of fermentation, the presence of *T. delbrueckii* contained the wild yeasts, mainly belonging to the genus *Hanseniaspora*, preventing their increase. Also, from an aromatic point of view, the wines inoculated with *T. delbrueckii* were characterized by an increase in isoamyl acetate, isobutanol and amyl and isoamyl alcohols. These results were supported by sensory analysis in which expert tasters gave a positive opinion and wines co-inoculated with *T. delbrueckii* obtained significantly better scores than those inoculated with only *S. cerevisiae*.

P27

Application of non-thermal technologies for the recovery of β-glucans from yeast biomass

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The food packaging industry is becoming increasingly aware of the hidden problems of plastic packaging, including its inability to degrade or regenerate. For this reason, biodegradable alternatives to petroleum- based polymers are being developed for food packaging. Among alternative biopolymers, β-glucans from yeast cell walls are getting a lot of attention due to their specific properties, such as high apparent viscosity, water-holding capacity and stabilisation of emulsions. Although yeasts contain an appreciable amount of β- glucan (30–60 % of the dry weight), the recovery and isolation of this compound could be difficult due to thecompact and rigid cell wall.

In this context, the aim of this study was to evaluate the potential of high-pressure homogenisation (HPH) and pulsed electric field (PEF) treatments to favour the rupture of yeasts cell wall and the release and recovery of β-glucans from yeast biomass to obtain innovative biopolymers.

Specifically, different HPH (125 MPa for 3 passes) and PEF (5–25 kV/cm for 30–240 μs) treatments combined or not with heat treatments at 90 °C for 20 min were applied on biomasses of commercial bakery yeast. The dispersions obtained were characterized by observation at epifluorescence microscope and for carbohydrates, proteins and β-glucan content, in order to assess the effective cell wall rupture.

HPH treatment at 125 MPa for 3 passes showed high dispersibility indices of proteins (37.5 % ± 0.03) and carbohydrates (24.7 % ± 1.56) and a high release of β-glucans, indicating that this technique may be suitable the cell wall rupture without the need of additional thermal treatments. Also, the combination of PEF and heat treatments increased the release of β-glucans from yeast biomasses. Both HPH and PEF treatments allowed to obtained films with positive properties, excellent continuity, and homogeneity.

P28

Evaluation of the microbiota of Salami Milano during production process by NGS analysis

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The microbiota of two bathces of typical italian dry sausages (salami Milano), produced with starter, was monitored by 16s rRNA gene sequencing. 30 samples collected at time of 0, 5, 15, 23 and 35 days of ripening and after HPP treatment (600 MPa for 5 minutes) were analysed. The sequencing was carried out through Ion GeneStudio™ S5 System (Ion Torrent™ Thermo Fisher) and the library for sequencing was generated from V2, V3, V4, V6,7, V8 and V9 variable regions of ribosomal 16S rRNA gene. From the samples of the first and second batches 1809734 and 805282 reads respectively were obtained. Alpha Diversity index shows that 90% of bacterial populations were correctly described in all sample. In the meat batter a total of 25 genera were identified in the batch one and 7 genera in the batch two. The most rapresentative genera found were *Lactobacillus*, *Staphylococcus*, *Pediococcus*, *Lactococcus*, *Leuconostoc*, Brochothrix, Pseudomonas, *Acinetobacter*, *Photobacterium*, *Psychrobacter*, *Serratia* and *Shewanella*. Starting from the fermentation phase until the end of seasoning the *Lactobacillus*, *Staphylococcus* and *Pediococcus* genera becomes the dominant microbial genera while the spoilage microorganisms were inactivated. The species found at the end of the seasoning, in addition to starter's species (*L. curvatus*, *P. acidilactici*, *S. xylosus* and *S. carnosus*), *L. sakei*, *L. graminis*, *P. pentosaceus*, *P. lolii*, Leuconostoc carnosum and *Carnobacterium* sp. were identified. The samples treated with HPP process showed no differences with the untreated samples except for the identification of *Bacillus* genus, probably due to the initiating germination of spores after the treatment.
The application of metagenomics techinques allowed to broaden our knowledge of the microbial composition in a dynamic ecosystem such as salami.
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P29

New insights on the use of probiotic yeasts to be applied in fermentation consortia for the production of functional health drinks

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Recently, it was demonstrated that fermented food or beverage brings benefits to the consumer's diet, especially in food matrices in which the fermenting microorganisms remain alive. Based on the recent re-evaluation of yeasts with probiotic characteristics, this research has found the foundations. In this study, five yeast strains with probiotic aptitudes belonging to *Candida zeylanoides*, *Yarrowia lipolytica*, *Kluyveromyces lactis*, and *Debaryomyces hansenii* species were assessed in a defined consortium, in co-culture with a commercial strain of *Lactobacillus casei*, to evaluate the yeasts' fermentation performance for healthy beverage. A stable consortium was obtained and all yeasts remained viable for five weeks at 4 °C, reaching about 8.00 Log CFU in 150 mL of trials, a volume corresponding to a pot of a commercial product. It is well established that yeasts consortium showed an appropriate fermentation performance also conferring peculiar and distinctive analytical and aromatic properties to final beverages, confirmed by a pleasant taste. These results allow us to propose the yeasts consortium as a versatile and promising multistarter tool, able to affect industrial fermented beverage market with both recognizable organoleptic properties and probiotic aptitudes.

P30

Grape treatments with *Starmerella bacillaris* and chitosan as a sustainable strategy to control *Botrytis cinerea* during the witheringprocess

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The reduction of chemical pesticides in the agri-food sector is a very topical issue. Environmental and health concerns related to the exposure or consumption of these substances are increasingly worrying the public opinion. The fruit post-harvest management is an important step where strategies can be developed to prevent pesticides from reaching the consumers table. In this context, the use of microorganisms as biocontrol agents seems a possible solution.

In this work, the yeast *Starmerella bacillaris* has been applied on drying grapes as a biocontrol agent against*Botrytis cinerea*, together with chitosan. The test was performed on two different grape varieties: *Garganega* and *Raboso*. During the withering process, the trend of the population of *S. bacillaris* released on grapes andthe growth dynamics of *B. cinerea* have been monitored by real-time quantitative PCR (qPCR) to observe changes related to the different treatments. Moreover, to compare the microbial biodiversity associated withgrapes surface and to verify if and how this can be influenced by the different treatments, DNA metabarcoding was used. Finally, at the end of the withering process grapes were pressed and the must fermented to evaluate the effect on alcoholic fermentation of the yeast *Starmerella bacillaris*.

A qPCR-based method for the identification and quantification of the selected *S. bacillaris* strain was successfully developed. The use of DNA metabarcoding provides a picture of the fungal biodiversity associated with the surface of the bunches. The application of *S. bacillaris* and chitosan caused an increase inthe biodiversity of fungal communities present on the surface of the *Raboso* bunches, together with the decrease in the proportions of filamentous fungi such as *B. cinerea*. This result was confirmed also by qPCR method demonstrating that, in the *Raboso* bunches, the application of *S. bacillaris* and chitosan inhibited the growth of *B. cinerea*.

P31

Genome engineering approach to evaluate the pathogenicity potential of STEC O174 strain from dairy environment

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In the last years, the number of Shiga toxin-producing *Escherichia coli* (STEC) outbreaks are increasingly linked to the consumption of dairy products, in particular those made with unpasteurized milk. Despite many efforts to study the pathogenicity of STEC, few information is available about the mechanisms of virulence mechanisms and of survival in food are currently available. Hence, the genome of the STEC strain UC4224 isolated from a semi hard raw milk cheese was sequenced and characterized, highlighting the presence of both the Shiga toxins stx1 and stx2 and the Locus of Adhesion and Aggregation pathogenicity island (LAA- PAI), which carried a collection of additional virulence factors. With the aim to investigate the virulence potential and create a strain with a reduced pathogenicity to be used as surrogate in challenge tests, three mutants lacking one and both *stx* genes, were created with a lambda red recombinase-based genome engineering approach. Their lethality was assessed in *Galleria mellonella*, used as *in vivo* model to study host–microbe interactions, revealing a significant decrease of mortality for both single and double mutants. Indeed, lethal doses (LD50) of 81.7 CFU/10 µl and 50.5 CFU/10 µl for single mutants and 582.7 CFU/10 µl for double mutant, respect to the 6.0 CFU/10 µl for the parental strain were observed. Larvae injected with negative controls showed no mortality. When tested in cheese model, mutant strains showed a similar replication capacity and a moderate resistance to lactic acid in comparison with the parental strain.
Overall, our results demonstrated that the removal of *stx* genes was not enough to abolish the lethality of the UC4224 strain, leading to suppose that its virulence has a multifactorial nature, and emphasizing the need to deepen knowledge on virulence potential of emerging non-O157 STEC serotypes in dairy products.

P32

Optimization of DNA extraction protocols compatible with high-throughput sequencing to study the microbial ecology of green coffee beans

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Coffee production is a long process consisting of several stages that affect the microbiota and the quality of the coffee itself. Agricultural and farm practices, which depend on the coffee plant species, geography, weather conditions, and available infrastructure, as well as the post-harvest processing method used, can affect the quality and the microbiota of green coffee beans. The microbial ecology evolves during the different stages of coffee processing and includes Acetic Acid Bacteria (AAB), bacilli, Lactic Acid Bacteria (LAB), yeasts, and filamentous fungi. Each microbial group in green coffee beans can be associated with different functionalities, and the type of microbiota present could be used as an indicator of origin. The aim of this study was to optimize DNA extraction protocols compatible with high-throughput sequencing to study the whole microbial ecology of green coffee beans. Variations in stirring time for cell detachment, volume used for extraction, and homogenization step were evaluated to obtain an efficient sampling procedure. Once the sampling procedures were standardized, 4 coffee samples of different geographic origin (Colombia, Vietnam, Uganda, Brazil) were tested to compare three different extraction methods: an in-house protocol and two commercial kits. The DNA obtained from all procedures was quantified and the effect of the extraction conditions on the microbial community of green coffee beans was monitored using PCR. Finally, samples from the two extraction methods were used for high-throughput sequencing. The importance of this study is to optimize and standardize a DNA extraction protocol for high-throughput sequencing in order to improve information about the microbial ecology of green coffee beans, which could be used as an indicator of origin.

P33

In vitro rumen fermentation traits of *Mentha piperita* and *Rose*

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From the point of view of the circular economy, the organic waste, obtained after the extraction of the essential oil, could constitute an important recovery material due to their content of bioactive compoundsand used as a supplement in the diet of dairy cows.
The aim of this study was to evaluate the in vitro rumen fermentable characteristics of *Mentha piperita* and *Rose*, used as the residual product obtained from a supercritical CO_2 extraction (Mint), and as the residual ofnormal extraction with glycol (*Rose*). Each fermentation was tested in three repeated incubations, and each incubation was carried on by using rumen fluids collected from three different dairy cows. A bottle containing only the medium, one containing only the rumen fluid, one containing grass hay supplementation, and one containing grass hay plus glycol were used as controls. Each bottle was fermented for 24 hours.
Following this analysis, microbial DNA from rumen samples was extracted with a commercial kit andsequenced by Illumina NGS methodology.
The different supplementations have led to the creation of different microbiota in each sample.
It was possible to observe how the microbiota varied according to the method used for the essential oil extraction. In particular, all the samples collected from the bottles containing glycol (the fermentation from *Rose* conventional extraction and related CTRLs fermentations added with glycole) *Anaerovibrio* taxa was always present with significantly higher abundances (about 40 %). *Anaerovibrio* are known to be involved inthe first step of ruminal lipid metabolism: the hydrolysis of esterified fatty acids to the free (non esterified) form and glycerol with little accumulation of mono- or diacylglycerols. The glycerol produced in this step is usually rapidly fermented from other ruminal bacteria to produce propionate. In progress are the fatty acids analysis to confirm this microbial processes.

P34

Promoting Innovation of ferMENTed fOods (PIMENTO) - COSTACTION CA20128

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Present in all European diets, fermented foods (FF) hold a strategic place due to the benefits they offer in terms of nutrition, sustainability, innovation, cultural heritage and consumer interest. The potential of FF for improving human health but also driving food innovation and local production in the next decades has become highly relevant. PIMENTO project, a COST Action CA20128 (Promoting Innovation of ferMENTed fOods; https://fermentedfoods.eu/), which started in November 2021, is supported by COST (European Cooperation in Science and Technology; www.cost.eu). The challenge of PIMENTO is to federate the scientific community and other key stakeholders working on FF. The long-term goal of PIMENTO is to place Europe at the spearhead of innovation on microbial foods, promoting health, regional diversity, and local production at different scales, contributing to economic and societal development as well as food sovereignty in order to promote multi-modal innovation and respond to the expectations of Europeancommunities.
The wide variety of stakeholders engaged will enable CA PIMENTO:
i) to tightly connect and clarify scientific knowledge on health aspects of FF
ii) to tackle technical, societal and legislative bottlenecks behind FF-based innovations
iii) to contribute to the establishment of long-term scientific workplaces
iv) to disseminate widely defined scientific knowledge on FF
v) to outline a strategic roadmap for future joint research.
PIMENTO will contribute to the European Green deal strategy “Farm to Fork” by enhancing research and innovation in fermentation-based solutions for food products and processes, improving nutritional, sensory and functional properties. This collaborative network of researchers that includes food scientists, innovators, entrepreneurs, microbiologists, biochemists, and nutritionists has a very broad geographical coverage with 396 partners from 283 institutions of 50 countries. This regional diversity will play an important role through considering a contrasted panel of FF in diets.

P35

EPS-producing *Lactiplantibacillus plantarum* as a tool to improveantioxidant activity of fermented milks

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Exopolysaccharides (EPS) producing Lactic Acid Bacteria (LAB) have been claimed to exert various health benefits to the host, including the ability to face oxidative and inflammatory related stress. This study investigated the ability of selected food-associated *Lactiplantibacillus (Lpb.) plantarum* to improve the antioxidant activity of fermented milks by producing EPS. Two *Lpb. plantarum* strains, properly selected as low- and high- EPS producers have been applied in a lab-scale fermented milks production, in combination with *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*, as conventional starters. The antioxidant activity of EPS produced during fermentation have been investigated *in vitro* using the DPPH, ABTS and FRAP assays, while the ability to modulate reactive oxygen species (ROS) level was evaluated in a healthy intestinal epithelium cell model (NCM460), subjected to both oxidative and inflammatory stress. Furthermore, to verify whether digestion affects their functionality, fermented milks were evaluated before and after an *in vitro* simulated gastro-duodenal digestion. The overall results showed that fermented milks produced with the *Lpb. plantarum* strain (LT100), the highest EPS producer, was endowed with a greater antioxidant capacity compared both to a commercial yogurt and fermented milks produced with the low-EPS producer strain, showing a strict correlation with the major production of EPS. Interestingly, the data showed a differential modulation of ROS production by intestinal cells upon either inflamed or oxidative stress with a protective anti-inflammatory effect *via* a significant reduction in ROS release by fermented milks enriched with *Lpb. plantarum* strains. Our data suggest that the use of selected EPS-producing *Lpb. plantarum* strains can be a promising natural strategy to enrich the functionality of fermented milks and/or yogurt in terms of ROS modulation and inflammatory related stress at intestinal level.

P36

Use of Lactic Acid Bacteria to reduce allergenicity of insect proteins

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Climate change and population growth are driving the search for alternative feed and food more sustainablethan conventional sources. In this context, new protein sources, such as insects, could be introduced into thefood chain, raising many health issues, especially in the field of allergies. Food allergy is an abnormal IgE- mediated response to otherwise harmless food proteins, affecting 5-10 % of children and 1-5 % of adults. Therefore, a significant challenge for global food health and safety concerns the study of new strategies toreduce food allergenicity.
Fermentation by Lactic Acid Bacteria (LAB) can represent an excellent solution since it allows to modify the structure of proteins and consequently their allergenic properties. In fact, thanks to a proteolytic system composed of cell envelope proteinases (CEP) LAB can degrade proteins into oligopeptides, deactivating theIgE-epitopes.
The purpose of this work is to investigate the proteolytic activity of LAB strains with complete genome aimed at the degradation of insect allergens. Based on the sequence of the predicted epitopes of insect proteins, an *in silico* analysis showed that the cleavage specificity of the CEP proteases PrtP and PrtR couldlead to the inactivation of these allergens.
The complete genome of 5 LAB strains (*Lacticaseibacillus rhamnosus* 1019 and 1473, *Lacticaseibacillus paracasei* 2306 and 2333, *Lacticaseibacillus casei* 4186) were then searched by using PrtP and PrtR sequences as queries. The results show that these proteases are diffused in the analysed LAB and different strains probably possess functional PrtP and PrtR. To verify the activity of the CEPs of the selected LAB, *invitro* tests are required by applying different growth conditions (time, temperature, concentration of *inocula*, etc.) in the presence of the insect allergens.
In conclusion, the application of LAB to reduce food allergenicity could represent a potential step forward inaddressing food allergies.

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P37

Elucitadion of the mechanisms of action of biological control agents against phytopathogens fungi

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Biocontrol agents have gained an important role in modern agriculture as they can be considered sustainable alternative or complements to agrochemicals in food production. Selected and tested biocontrol agents can reduce or prevent the proliferation of plant pathogenic fungi, through the synthesis of antimicrobial compounds, lytic enzymes and/or inducing systemic resistance. In the present study five *Pseudomonas* candidates biocontrol strains (*P. putida* PSP1, *P. putid*a PZY6, *P. fluorescens* PF05, *P. fluorescens* PF4.89 and *P. protegens* PSPR04) were tested to evaluate their ability to produce lytic enzymes such as proteases, lipases, cellulases and amylases in different medias (skim milk, nutrient agar supplemented with egg yolk, nutrient agar with starch and M9 with CMC).

After incubation, halo formation around the colonies was evaluated to measure and rank the five strains based on their enzyme-producing performance. All of the tested isolates were able to grow in different test medias containing specific substrates as the major carbon sources. In particular, strain PF05 was identified as the most proteolytic while PZY6 was best for lipase activity. Amylases production was more pronounced in PSP1 while PSPR04 performed best for cellulase production.

Further studies are now being performed to evaluate interactions between candidate biocontrol strains and specific plant pathogens. Bacterial antagonism against fungal/oomycete phytopathogens such as *Fusarium*, *Botrytis*, *Pythium* and *Rhizoctonia* is studied through dual culture plate assay, together with RNA-seq analyses, specifically targeting genes and pathways involved in the biocontrol activity of *Pseudomonas*.

P38

ZERO WASTE: hemp wastes as a source of biotechnological usefulproducts for yeast and human cell cultures

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The hemp industry produces large amounts of wastes (flowers, fibers and leaves) treated as materials with a negative impact in terms of industrial sustainability due to the high costs for their management. In an attempt of sustainable agriculture and circular economy-based model, a good alternative may be represented by the reuse of these wastes as a source of useful compounds. *Cannabis sativa* L. plant is known for the high- quality oil, carbohydrates, fibers, vitamins, mineral elements and protein. In particular, the protein fraction is appreciated for its nutritional value, industrial and scientific interest. In this framework, aim of this study is to produce different Hemp Extracts (HEs) from hemp wastes (*Cannabis sativa* L.) by using chemical or physical hydrolysis together with short and long-term microbial degradation. Each HE was characterized for the chemical-physical properties as well as for the protein content. HEs were tested at increasing concentration up to 1 mg/mL for their ability to support both yeast and human cell cultures growth, also evaluating their cytotoxicity and antioxidant effect. A FTIR-based bioassay was employed to investigate the stress-induced cell status in response to the HEs addiction. The results showed that the effect exerted by HEs is mainly affected by the tested concentrations. At the lowest protein concentration, hemp extracts were able to support the growth of yeast and human cell cultures without any toxic effect. Furthermore, HEs were able to significantly reduce the lag phase of growth of yeast cells. Conversely, at the highest concentrations, HEs depress cells growth showing a strong cytotoxic effect. Although further studies are necessary to confirm these results, the data obtained confirm that hemp wastes may be upgraded to useful products by means of cost-friendly and microbiological-aided processes representing a valid eco-sustainable alternative in their management.

P39

Lactobacillus delbrueckii subsp. *bulgaricus* derivatives embedded inalginate/hyaluronic acid self-crosslinking hydrogels: an analyticalcharacterization, 3D printing manufacturing and *in vitro* wound healing potential

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Many variables and collective biological mechanisms are involved in the complex wound healing processes. The development and application of innovative biomaterials as advanced therapeutic solutions including sustainable natural derivatives are constantly evolving in a huge challenging fashion.
This work aims, through a critical discussion, to add insights into the therapeutic potentials in skin repair of functional 3D printed biomaterials mimicking the natural extracellular matrix (alginate/ALG and hyaluronic acid/HA) and manufactured as hydrogel embedding *Lactobacillus delbrueckii* subsp. *bulgaricus* (LB) derivatives (proteins, lipids, and exopolysaccharides (EPS)). A comparative study of the behavior of two different strains, LB 1865 and LB 1932 differing in the EPS production was performed.
A stepwise comprehensive analytical characterization of these derivatives was allowed using LC-MS/MS, UV/VIS, ATR-FTIR and SEM techniques. The formulation of innovative self-crosslinking inks for 3D printing extrusion technology based on ALG, ALG/HA and ALG/HA/LB derivatives mixtures consented the accurate embedding of the lipophilic and hydrophilic extracts in the highly structured hydrogels without any time-consuming and poorly reliable post-processing loading step.
In a series of *in vitro* tests on human fibroblasts attention was captured by the EPS that exhibited a relevant dose-dependent cytocompatibility.
The LB derivatives showed an ability to increase cell proliferation and migration, quantifiable between 10 and 20 % if compared to controls, with higher values for the derivatives obtained from the LB 1932 strain. These findings were in correlation with an observed decrease in matrix-degrading and proapoptotic proteins, together with an increase in factors involved in the healing process, collagen and antiapoptotic proteins production.
Finally, a brief overview on the future perspectives of this research will be exposed.

P40

Improving isolation of Next-Generation Probiotics strains from human gut optimizingculture media and enrichment procedures

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The term Next-Generation Probiotics (NGPs) refers to microbial strains that can have a positive effect on human health, but do not belong to common probiotic species (e.g., Lactic Acid Bacteria, *Bifidobacterium*). However, several studies highlighted the unexplored source of potentially beneficial microbes in the gut microbiome. For this reason, academic and industrial research is focused on identifying and testing novel microbial strains of gut origin. One of the main issues in NGP culturing was the selection of a suitable culture medium that allowed the growth of these high-demanding species. Consequently, we tested nine culture media with different formulations in terms of vitamins, minerals and fatty acids to study the culturable fraction of the gut microbiome. We collected bulk microbial cells grown on two plates of each medium and analyzed them by 16S rRNA sequencing of the V3-V4 regions. In addition, we sequenced amplicons obtained from the original fecal samples to identify differences among the media and which of them gives a more reliable picture of the gut microbiome. Results obtained allowed us to select four media that supported the growth of the highest number of putative NGP species. Samples were streaked on four media and incubated in aerobic and anaerobic conditions at 37 °C to discard facultative anaerobes. To select strict anaerobes, we also tested a pre-enrichment step of fecal samples in different broths for 48 h in anaerobic conditions. Fifty-two bacterial colonies were isolated and identified, including the promising NGPcandidate *Bacteroides uniformis*.

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P41

Legumes by-products: fermentation as a strategy to discover newantimicrobial compounds

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Legumes are largely consumed because of their nutritional value and their production increased in the last years. During industrial processing, up to 25 % of the total production is wasted, generating a huge amount ofwaste and by-products, that are still rich in value-added compounds. However, the actual management strategies of legumes by-products are not considered sustainable, and fermentation can be a new and effective strategy for their recovery. This low-cost bioprocess aims to valorize by-products by improving their nutritional value or through the production of biologically active compounds.

Based on this background, this work focused on the fermentation of legume by-products to produce biomasses characterized by high antimicrobial activity against food-borne pathogens. Fermentation was carried out using the species *Lactiplantibacillus plantarum*, *Lacticaseibacillus rhamnosus* and *Bacillus subtilis*, and ten different matrices related to legumes by-products were tested: peas, chickpeas and beans by- products, protein extracts obtained from them, the residual of the extractions, and bean hulls. Successively, the antimicrobial activity of the fermented by-products was tested by microbial challenge test against *Salmonella enterica*, *Listeria monocytogens*, *Staphylococcus aureus*, and *Escherichia coli*.

To evaluate how matrices, starters microorganisms, fermentation temperature and incubation time affected the fermentability of these matrices and the antimicrobial activity of the same fermented by-products against the different pathogens, an experimental design has been planned. The results highlighted that *L. rhamnosus* had the best growth ability on these substrates but not the best inhibitory effect, that instead was observed especially against *L. monocytogens* with samples fermented by *L. plantarum*. Antimicrobial activity was related to the fermentation temperature but not to the incubation time. Considering the lack of similar studiesin the literature, these preliminary results could open new perspectives in the recovery of legumes by- products by fermentation and the development of natural antimicrobials using this bioprocess.

P42

“Smart” fermentations to improve sustainability of food supplychains by blending side streams

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Transforming food by-products, especially those containing an attractive level of proteins, fibres and polyphenols, into affordable ingredients for new products is a promising and responsible strategy for achieving sustainability in food supply chains. This requires a rethinking of the organization of food processes and the adoption of innovative approaches that can connect material flows that were not previously considered.
The FERBLEND project (https://susfood-db-era.net/main/FERBLEND) aims to address this by valorizing two side streams from oilseed processing and dairy industry through fermentation and creating platform products with improved technological and nutritional functionality. Likewise, it is important to ensure that appropriate measures are taken to maintain food safety and sensory quality in these substrates. This approach not only reduces waste but also provides an opportunity for innovation in the food industry: the resulting fermented side streams blends can be used in a variety of end products, from beverages to baked goods and snacks. Small and medium-sized enterprises face challenges in handling and downstream processing of side streams, which can be addressed by the strategies developed in this project.
Two models, controlled and spontaneous fermentation of blends with different compositions and residual contamination were studied. In the first model, microcosms made with sweet whey and sunflower press cake, inoculated with different combinations of previously selected strains (mesophilic LAB and lactose- fermenting yeasts) were investigated. In the second model, acid whey naturally containing lactococci was used as liquid component and suitable microbial *inoculum*. A back-slopping technique was performed to shape and stabilize the microbial populations exploiting an adaptive evolution phenomenon. Culture- dependent and -independent techniques were applied and the obtained results were both consistent. The outcomes of the work can provide valuable insights for developing innovative processes and materials to manage food side streams and implement the circularity of food resources.

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Microbial fermentation to improve the nutraceutical value of pâté olive cake

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Recently, growing attention has been focused on the valorisation of olive pâté (POC), a by-product obtained from the extraction of olive oil through the multiphase decanter (DMF). This by-product is characterized by a high moisture content, the absence of kernel parts and the presence of bioactive compounds, thus representing a matrix suitable for the formulation of new functional foods. The aim of the present study was to stabilize and improve the nutraceutical value of POC through driven fermentation using three microbial strains in single cultures *Lactiplantibacillus plantarum*, *Wickerhamomyces anomalus* and *Candida boidinii*, previously isolated from fermented table olive brines. In detail the strains were inoculated at 1 %, at an initial cell density of 10^8 CFU/mL and 10^7 CFU/mL for *L. plantarum* and the two yeasts, respectively. All trials were performed in a bioreactor in a volume of 3 L of POC. Chemical, microbiological and molecular analyses were carried out at the beginning and at the end of fermentation. In addition, all POC samples were analyzed at the end of fermentation for antioxidant activity. The results showed that the lowest pH value (4.09) was reached after 10 days in sample inoculated with *C. boidinii*. Microbiological analyses exhibited high yeast cell densities throughout the whole process with a value ranged from 5.5 to 7.80 Log CFU/g, as confirmed by PCR-DGGE profile. The acetic acid content, evaluated by HPLC, increased greatly in all samples, reaching the highest concentration (13048.7 mg/L) in the control sample. Regarding the phenol content, a decrease in hydroxytyrosol was observed in all samples at the end of fermentation, although the highest concentration was found in the fermented samples. Finally, the best antioxidant activity was obtainedin the sample inoculated with *W. anomalus*, where a percentage of radical scavenging activity ranged from 34.87 to 48.53%.

P44

Alternative preservatives tools to promote healthier traditionalfermented sausages

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Fermented sausages are meat products that have a long tradition in the Mediterranean diet. The preservation of these products is obtained by the fermentation process and the use of nitrates and nitrites. Although these compounds are used for their abilities to reduce pathogen incidence. However, their usage has been linked topotential health risks for the human health. The aim of this study was to find alternative strategies, like the addition of bioprotective starter cultures and vegetable extracts, in order to reduce the current use of chemicals. Autochthonous bacteriocin-producing lactic acid bacteria were characterized from naturally fermented sausages while specific botanic extracts were selected and screened for their minimal inhibitory concentration (MIC). Bacteria and extracts were screening in situ for their potential antimicrobial activities against *Listeria monocytogenes, Staphylococcus aureus* and *Clostridium sporogenes*. *Latilactobacillus curvatus* and thyme extract display the best combination and were then used in a pilot study with inoculated fermented sausages. The alternative preservatives showed to reduce the incidence of *L. monocytogenes* and *S. aureus*. The findings of this study open new possibilities regarding the implementation of these alternativepreservatives for the meat industry.

P45

Characterization of the microbiota of Parma ham by Next-Generation Sequencing technology

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The Next Generation Sequencing (NGS) technology was applied to investigate the microbiota of ParmaHam.
A total of 100 Parma hams 24-months old were collected from 10 plants located within the geographical boundaries of the Parma production area. The sequencing was carried out through Ion GeneStudio™ S5 System (Ion Torrent™ Thermo Fisher) and the library for sequencing was generated from V2, V3, V4, V6, V7,V8 and V9 variable regions of ribosomal 16S rRNA gene. A total of 5,196,943 operational taxonomic units (OTUs) were obtained for genera and 4,091,845 for species. A total of 68 genera and 93 species were identified, distributed in a highly variable manner depending on the plant of origin. The results obtained were then processed using the nonparametric Kruskal-Wallis statistical test, which revealed the presence of significant differences in the relative abundances of the various genera among the production plants. The dominant microorganisms were found to belong to the genera of *Tetragenococcus*, *Staphylococcus*, *Lactobacillus*, *Brevibacterium* and *Corynebacterium*; the combined comparison of all samples only revealed two genera that were shared by all plants, Tetragenococcus and *Staphylococcus*. Not a single characteristic species of a production plant was identified; on the contrary, it was possible to recognise the presence of *Tetragenococcus halophilus* in all 100 samples.
Further experiments are needed to elucidate the role of those microorganisms that could have the highestimpact on the formation of the characteristic colour and flavour of Parma Ham, a typical dry cured ham produced without nitrate or nitrite.
Overcoming the constraints of traditional methods, NGS technology has proved to be an indispensable toolfor enhancing knowledge of the microbial composition of this product.

P46

Recycling wastes from *Rubus idaeus* by-products: sourdough breadproduction as a new end-use of exhausted seeds still containing active compounds

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In the past few years, in light of the European Commission's directives on environmental sustainability, the food industries and academic research institutes focused their attention on the use of plant waste and by- products as ingredients to improve the nutritional value of foods. In this work, the agro-industrial waste fromcold pressing of *Rubus ideaus* seeds (WRS) was explored as a valuable food ingredient in bread production. Bread making trials were performed under laboratory conditions using a mix of wheat flour and semolina (1/1) and sourdough as fermenting agent. Two experimental bread productions (5-WRS and 10-WRS) were obtained replacing wheat flour/semolina with WRS at 5 and 10 % (w/w), while control production (CTR) was WRS free. WRS did not negatively affect sourdough LAB starter development and their cell densities reached almost 10^8 CFU/g at the end of fermentation. Illumina technology identified 14 taxonomic groups, and lactobacilli constituted the major group of the mature sourdough (75.91 % of relative abundance (RA)) and doughs (82.08 – 88.76 % RA). WRS decreased the volume and increased crust and crumb redness of the final breads. The replacement of wheat flour/semolina with WRS at 5 and 10 % (w/w) significantly increased the functional value of breads, in term of content of polyphenolic compounds, proanthocyanidins content and antioxidant activity. In particular, these functional properties increased until 25 % in bread, confirming the thermal stability of WRS. The addition of WRS did not spoil the sensory traits of breads, but the highest values of overall satisfaction were displayed by 5 % (w/w) WRS enriched breads. This work clearly indicatedthat the addition of WRS in bread production represents a promising strategy to increase the antioxidant activity in cereal-based fermented products.

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P47

The implication of *Lactobacillus helveticus* strains isolated fromnatural whey starter in methylglyoxal-mediated browning

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Reducing the processing waste and preventing possible defects in dairy industry represent a valuable way to avoid economic losses. In this regard, Grana, like other hard cheeses, can develop browning during ripening, a defect of concern because of its implications on appearance, texture, and flavour of the final product. The browning process involves the formation of methylglyoxal by the microbial community that results in late- stage reaction compounds typical of the Maillard reaction in presence of the products of proteolysis. In this work, a rennet casein whey medium was employed to shed light on the strain-specific *L. helveticus* ability to produce methylglyoxal and discoloration, by combining colorimetric assays, real time-PCR, and high- resolution mass spectrometry. Thereafter, an untargeted metabolomics approach based on ultra-high- pressure liquid chromatography coupled to high-resolution mass spectrometry was used to unravel the methylglyoxal downstream reactions and late-stage products, to support the presence of compounds associated with browning discoloration. The results corroborated that methylglyoxal production is a strain- specific feature among the different *L. helveticus* isolates. Concerning the browning process, metabolomics strengthened the involvement of pyrazines and β-carboline compounds, particularly at the earlier stage.

Thereafter, the reduction of methylglyoxal to 1,2-propanediol has been postulated to be a major detoxification route in methylglyoxal-producing strains, together with the conjugation to form S-Lactoylglutathione via the glyoxalase-pathway. Nonetheless, looking at both earlier and later stages of the in vitro protocol adopted, the essential role of free amino acids and dipeptides arising from proteolysis has been highlighted. Together with pointing out the importance of specific *Lactobacillus* strains in hard cheese discoloration, our data corroborate the need for different co-occurring factors to develop browning, thus explaining the sporadic appearance of the process. From a more practical point of view, our results suggest the need for more careful evaluations of natural starters used in traditional cheese production.

P48

The gill-associated microbiome in intertidal mangrove brachyurancrabs

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Microbial symbioses play a major role in the evolutionary history of both eukaryotic hosts and the associated microbiota. Because of their adaptability, metabolic flexibility and resilience to environmental changes, prokaryotic symbionts can support host adaptation to challenging conditions and environmental constraints. Given their density, species diversity and ecological functions, brachyuran crabs are considered one of the most important faunal groups of the mangrove ecosystems. They have variable levels of intertidal adaptations and occupy different ecological niches along the sea-land boundary and the occurring tidal range. In these animals, the gills finely regulate multiple physiological functions both under tidal submersions and emersions, effectively adapting to the challenges posed by the contrasting chemical- physical conditions of air and water.

We hypothesize that mangrove crab gills are consistently colonized by prokaryotes that may qualitatively and quantitatively change along the intertidal gradient supporting the host's adaptability to overcome the dielchallenges posed by tidal variations.

Taking advantage of both molecular techniques and electron microscopy, we verified the stable colonization of microbes on the gill surface and analyzed the gill microbiota among mangrove crabs occupying different intertidal ecological niches. Both electronic microscopy quantification and real-time PCR techniques showed that microbial abundance had a declining trend with the increase in tidal levels. Moreover, crab species occupying different tidal levels harboured different bacterial compositions and bacterial cell morphologies on the gill surface. Alpha diversity of gill-associated bacteria had a decreasing tendency among species fromsubtidal to supratidal zones.

Our findings point out a tight prokaryote partnership with the crab gills, with potential positive effects onanimal adaptation to the changing conditions along the intertidal gradient in the mangrove ecosystem.

P49

Plasma Activated Water to improve safety and sustainability of leafy vegetables production

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Recently, the attention paid both by consumers and the legislative body towards sustainability of food processing has increased. One of the key phases for ready-to-eat vegetables is the sanitization process by washing with chemicals e.g. chlorinated compounds, which are recognised to be effective against pathogens and spoilage microbiota, but potentially harmful to the consumers and their ecosystem. Also, decontamination of wash waters is generally achieved through chemicals including hypochlorite. Possible alternatives must be considered based on cost-effectiveness, sustainability and possible side effects on product characteristics. Non-thermal treatments based on cold plasma technology, e.g. Plasma Activated Water (PAW), are an emerging alternative. In this study, the decontamination ability of PAW on indigenousmicrobiota and target pathogens of rocket leaves and wash waters was tested. Processing of wash water targeted *Escherichia coli*, *Salmonella enteritidis*, *Listeria monocytogenes*. Decontamination of rocket samples was performed by dipping into PAW under stirring for different times (0, 10, 20, 30 minutes) followed by spin drying. Vegetable samples were analysed immediately after treatments and storage at 4 °C. Differences in sensitiveness were detected in relation to the pathogenic species and the organic load of wash waters. 20 and 30 minutes presented the highest decontamination effects on rocket leaves, with reductions of ~2 Log CFU/g for Enterobacteriaceae and 1.7 Log CFU/g for psychrophilic and mesophilic bacteria. Such treatments also reduced their growth during storage resulting in cell counts 1 Log lower than the control vegetable. PAW treatments showed efficacy in reducing the main indigenous microbiota without affectingthe visual quality of the product.

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P50

The cocoa bean fermentation method modulates the bacterial andfungal communities

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As the fermentation of cocoa beans is spontaneous, unpredictable, and conducted in different production areas following various methods in conditions not always well controlled, the resulting final quality is variable. From a microbial perspective, controlled fermentation aims to accelerate the process, increase production and minimize waste and unexpected results in an environmentally sustainable manner, while also influencing the fermentation to achieve a consistent, standardized final quality. Cocoa demand is growing by the day but, on the other hand, climate change, which affects cocoa plantations, decreases productivity.
This research aimed to examine the impact of three fermentation methods (box, ground, and jute) on the microbial populations in cocoa beans using High-Throughput Sequencing (HTS) of phylogenetic amplicons. Furthermore, the preferable fermentation method was evaluated based on the observed microbial dynamics. The results showed higher bacterial species diversity for beans processed in boxes, while ground fermentation had a wider fungal community. *Lactobacillus fermentum* and *Pichia kudriavzevii* were present in all three fermentation methods studied, while *Acetobacter tropicalis* dominated in box fermentation and *Pseudomonas fluorescens* in ground-fermented samples. The most relevant yeast in jute and box was *Hanseniaspora opuntiae*, while *Saccharomyces cerevisiae* prevailed in the box and ground fermentation. Potential interesting pathways were identified using PICRUST analysis. Overall, the study revealed significant differences among the three fermentation methods. The box method was found to be the most preferable due to its limited microbial diversity and the presence of microorganisms that ensure a good fermentation. Moreover, the present study allowed us to conduct a comprehensive examination of the microbiota present in cocoa beans treated with different methods and to better understand the technologicalprocesses useful to obtain a standardized final product.

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Soil microbial functions and biodiversity of Evolutionary Populations: the results of CHANGE UP project

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Intense annual crop production is causing a global exacerbation of natural resources degradation and biodiversity loss leading to adverse impacts on essential ecosystem services. In addition, conventional crops are threatened by climate change that strongly impacts agricultural yields. Therefore, there is a need to adopt sustainable agricultural practices that could decrease the agricultural impacts on climate and enhance agriculture resilience.
Evolutionary Cereal Populations (EPs) possess a high degree of genetic diversity, thus holding an inherent higher buffering capacity than homogeneous varieties to adapt to various stresses. EPs are therefore well suited to low input and organic agriculture due to their ability to produce good and stable yields even when the availability of resources decreases or when climatic conditions become extreme. An important but underexplored aspect of the adaptation of EPs to grow in hostile environments is the cross-talk between root systems and microorganism, which determines soil processess and microbial functions.
The aim of this work was to investigate if EPs cultivation leads to contrasting soil functioning. To this end, we characterised chemical and microbial signatures of bulk and rhizosphere soils from EPs grown at two Italian sites (Parma and Rome) after different precessions (legumes and wheat). We performed Mid InfraRed Spectroscopy to obtain a fingerprint on the soil organic matter (OM) quality in the different systems. In addition, we determined enzymatic activities, microbial biomass, Soil EL-FAME (ester-linked fatty acid methyl ester), and elemental concentrations to link microbial activity to OM quality and soil chemical characteristics. Our results demonstrate that EPs can influence the soil organic matter composition through alteration of microbial functioning depending both on site and cultivation system. Next generation sequencing analyses on rhizosphere samples will be performed in the future to link soil processes to microbial community composition and abundance in the rhizosphere of EPs at different sites.

P52

Production of Itrana table olives using *Lactiplantibacillus pentosus*O17 as starter culture

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Fermented table olives are produced in several countries of Mediterranean area with a significant economicimpact. To date, the production of table olives is mainly based on spontaneous fermentations, although several studies have highlighted the benefits of using starter cultures (e.g., inhibition of spoilage and pathogen microorganisms, drupes de-bittering, improved organoleptic features).

In this study, a spontaneous fermentation (carried-out according to the production disciplinary of Italian Gaeta olives PDO; trial A) was compared with two driven fermentations (trials B and C) by using *Lactiplantibacillus pentosus* O17 as starter culture. In trials A and B, salt (7 % w/w) was added when the pHof brine dropped to 4.5 (as for Gaeta PDO); in trial C, salt was gradually (up to 7 % w/w) added right from the beginning of process. pH, titratable acidity, NaCl, total phenolic compounds (TPC) and different microbial groups (total bacteria, LAB, yeasts, moulds, Enterobacteriaceae, halophiles) were monitored, in both brines and olives, up to 6 months of fermentation.

Major changes were observed in the first month of fermentation, and were mainly related to the salt and starter additions. Compared to spontaneous process, *Lpb. pentosus* O17 led to a better acidification rate, and reduced the enterobacteria growth. Starter culture showed a satisfactory adhesion ability to olive surface.

TPC content was affected by both olives-to-brine diffusion and microbial metabolism. Over time, the osmotic pressure impaired the starter viability, and yeasts (more tolerant) dominated the last steps of Itranafermentations.

Our data suggested that *Lpb. pentosus* O17 maybe a suitable starter to improve the quality of Itrana olives. However, further studies are needed to clarify the role of yeasts during the fermentation process.

P53

Spatial Distribution and Fermentation Procedures of EuropeanPDO- and PGI-Cheeses

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Food products, which fulfil certain criteria may obtain the European quality labels of “Protected Designationof Origin” (PDO) and “Protected Geographic Indication” (PGI). Cheeses contribute in a large proportion to the EU PDO- and PGI product list and the labels guarantee high quality, and the reproducibility of the sensorial properties of the cheese by regulating production steps and microbial composition during fermentation.
I screened 238 European PDO- and PGI-cheese regulations in 21 countries in order to define the diffusion ofdifferent technological processes in the EU. The goal was to evaluate the prevalence of different practices, considering the use of natural and commercial starters, type of milk, and application of heat treatments.
On top of the list of EU countries with PDO-/PGI certified cheeses there are Italy and France with 55 cheeses each, followed by Spain with 30, and Greece with 23 registered cheeses. On the contrary, the lowestnumber of registered cheeses was identified in Belgium, Ireland, Cyprus, and Hungary (n=1). Bovine milk is used to produce most cheeses but also sheep and goat milk are employed for cheese manufacturing. Around 49 % of the cheeses are fermented using commercial starter cultures in combination with heat treatment, followed by 28 % which use optional starters and pasteurisation. Just a small proportion of the cheeses (around 10 %), are traditionally fermented from raw, unpasteurised milk with the addition of natural whey starters or without any starter cultures.
Traditional cheese fermentation, in contrast to the use of commercial starters, preserves the unique microbiome of the cheese, that is important to produce specific organoleptic traits typical for each cheese.Microbiome may be considered as a fingerprint of traditional cheeses, that might be useful to trace cheese origin. In addition, a high microbial diversity may have beneficial effects on the consumers´ health.

P54

Screening of yeast and Lactic Acid Bacteria for biotechnological valorisation of clementine residues into functional ingredients

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The citrus juice industry yearly generates large amounts of residues still containing high-value compounds which can be valorised by the food industry. The overall aim of this study was to set up a tailored biotechnological process, based on non-thermal pre-treatment (pulsed electric field) and microbial fermentation, to produce a functional ingredient from clementine juice by-products. Since their chemico- physical conditions (low pH, low level of fermentable sugars, richness in polyphenols and essential oils) can inhibit microbial growth, a preliminary screening of different microorganisms and strains performances was conducted. In particular, 12 Lactic Acid Bacteria (LAB) and 19 yeasts (inoculum ~7 and 5 Log CFU/g, respectively) were employed. Their growth ability in clementine residue/water medium, prebiotic activity, total phenolic content (TPC), antioxidant activity (ABTS•+) and volatile compounds (SPME/GC-MS) of the fermented residue were evaluated. Some yeasts showed growth after 24-48 hours, while others presented a prolonged lag phase and grew only after 5 days (overall mean cell increases 2-3 Log CFU/g). All the LAB survived after the first 3 days, with some strains keeping viability up to 6 days. An increase in the antioxidant activity was detected following fermentation with *S. cerevisiae* and *Y. lipolytica*, *L. casei* and
L. plantarum. Except for two samples, all the tested strains improved TPC values with species- and strain- related differences. Evaluation of the volatile compounds confirmed that microbial diversity positively influenced the aroma profile of the samples, which were enriched in terpenes (alpha-terpineol; terpinene-4-ol) and alcohols (ethyl acetate). Selecting microbial species and strains based also on the desired characteristics is essential to valorise the clementine by-products and its use as a functional ingredient or flavoring agent e.g. for beverages.

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P55

Crossover starter yeast strains for the production of Italian GrapeAle (IGA), a fruit beer brewed after grape must adding

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A new beer type known as Italian Grape Ale (IGA), a kind of link beverage between wine and beer nowadays represents a popular and trendy production choice among brewers in Italy. In this investigation, three autochthonous *Saccharomyces cerevisiae* strains (denoted CHE-3, P4, TA4-10), formerly isolated from different food niches, were evaluated for brewing in comparison with the commercial strain US-05.
Fermentations were carried out at a laboratory scale on malt extract added with 15 % or 25 % grape must and on malt wort alone (control). During fermentation, CHE-3, P4, TA4-10 strains produced CO_2 amounts significantly higher than US-05. The adding of the grape must improved the CO_2 production, particularly when the wort was supplement with 25 % of grape must. Both the grape must addition to the fermentation medium and the used strains intensely influenced several analytical parameters such as the profile of volatile compounds, they being related to the organoleptic quality of the produced beers. The highest aromatic was detected in the IGA with 15 % grape must addition. To the best of our knowledge, this investigation is the first one concerning the employment of the microbial cross-over for the production of IGA craft beer in Southern Italy. The results obtained showed that both the yeast strain and the fermentation substrate determined the process dynamics and physico-chemical features of IGA beers. We showed that yeast starter strains originating from other traditional food chains could brew high quality IGA beer, thus endorsing the potential of cross-over fermentation for the development of novel beverages. Moreover, these findings confirm that wild microorganisms, isolated from diverse niches and preserved in microbial collections, are a fundamental pool of novel starter cultures able to produce enhance foods and beverages differentiation.

P56

From kiwi by-products to high-value added compounds

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The European Green Deal sets ambitious objectives for the economy and in particular for the agricultural sector. One of the objectives is the creation of full circularity of systems agribusiness. This is one of the challenges of the National Center for the Development of New Technologies in Agriculture (Agritech) funding by the European Union, faced by spoke 8 “New models of circular economy in agriculture throughwaste valorization and recycling”.
Agriculture produces many waste and by-products that can be transformed into new products. Organic wastecontains valuable compounds and biomolecules that need to be valorized. To do this, new low-cost and sustainable approaches/technologies will be developed to obtain high-value components that can be reused, for example, as agricultural products, feed, food, and pharmaceuticals.
This work focused on the fermentation of residual biomasses and in particular took into consideration kiwi discarded in the field due to its size or shape or not sold on the market and different parts of the fruit were considered. Fermentation, with Lactic Acid Bacteria and yeasts, was chosen as a technique to valorize the substrate, and an experimental design (DoE) was created to allow the screening of different strains and different process conditions in order to identify the optimal one. Fermented products were analyzed for the aromatic profile, to identify the variation in aroma compounds, but also the production of antimicrobial compounds, which was tested by challenge test. The optimal condition for fermentation process, and the best starter to be use were identified and used to ferment kiwi biomass for a second step of the work, which was the employment of fermented biomasses as biofertilizer. This approach has allowed the evaluation of multiple ways to follow to valorize kiwi residual biomasses and preliminary results suggest that it possible obtain multiple compounds after fermentation.

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P57

Effect of various carbon sources on bacterial cellulose synthesis in *Komagataeibacter xylinus*: phenotypic assessment of *wild type* and Δ*gdh* K2G30 strains

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Some microorganisms can synthesize a biopolymer known as bacterial cellulose (BC), successfully appliedin various fields.
In this study, *wild type* (UMCC 2756) and Δ*gdh* (UMCC 3007) K2G30 strains, the latter resulting from membrane glucose dehydrogenase-encoding gene silencing, were grown on different substrates, to assess BC and gluconate production over time. Both strains were first grown in Hestrin-Scramm medium (HS) and a variant thereof, obtained by adding ethanol; BC and gluconate were assayed on the sixth and eleventh daysof growth, along with glucose and ethanol consumption. Next, strains were grown on alternative HS mediums, prepared by replacing glucose with mannitol, glycerol, and fructose. BC, gluconate production and substrate consumption were appraised on the fourth and seventh days of growth. Finally, milk whey wasused as substrate, and the quantification of BC and gluconate production was performed on the third, seventhand eleventh days of growth, along with lactose, lactic acid and galactose consumption.
Δ*gdh* strain didn't take advantage of ethanol supplementation to enhance BC yield, thus showing a lower glucose uptake in the earliest stages of growth compared to the *wild type*. Furthermore, Δ*gdh* showed an higher BC yield and trophic activity when grown on alternative substrates. Milk whey turned out to be a suitable growing substrate for both strains. We suggest that gene silencing affects both carbon source uptakeand BC synthesis in Δ*gdh*.

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P58

A model system to describe microbial interactions during the early stages of cheese manufacturing and ripening

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Grana Padano (GP) PDO cheese is a raw milk cheese manufactured using natural whey starter (NWS), an undefined microbial culture, which is produced daily by backslopping by each dairy. The microbial ecology of NWS has been investigated over the years, but few studies have dealt with the aspects related to microbial interactions. In this work, we evaluated 3 *Lactobacillus helveticus* (LH) and 3 *Lactobacillus delbrueckii* subsp. *lactis* (LD) strains, isolated by NWS and curd of GP, by measuring their growth performance in milk and a miniaturized cheese model. Binary cultures were made mixing one strain of each species, obtaining 9 different combinations. Each binary culture was assessed for its performance in the early stages of milk acidification by use of impedometric technique, while microbial dynamics were followed by plate counts and quantitative Real-Time PCR (qPCR). The 6 binary combinations showing the best acidification performances were used for the manufacturing of miniature cheese models, which allowed for simulating the cheese ripening, with four weeks of incubation, monitoring the microbial growth, and the production of volatile compounds by gas chromatography. The results revealed cooperation between the species LH and LD during milk acidification, leading to increased performances compared to the strains in a single culture. Model cheeses highlighted that the dynamics of the species LD and LH show similar trends, regardless of the isolates, with the species LD reaching higher cell concentrations compared to LH. Different strains' combinations led to different volatile profiles, indicating that complex microbial interactions can greatly affect the properties of the resulting product. Due to the complexity of the microbial ecosystem of raw-milk cheese produced with undefined cultures, the availability of a model system can provide an effective tool for testing starter cultures and characterizing autochthonous strains, to valorise the biodiversity of artisanal food manufacturing.

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Factors affecting food security in raw-milk pasta-filata cheese manufacture

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Despite research findings on the absence of pathogens in raw-milk cheeses, there is still debate about food security of traditional cheese production systems. This study aimed to evaluate the effect of stretching temperatures on the growth of the four dairy pathogens bacteria during raw-milk pasta-filata cheesemaking. Cheese production was carried out with raw cow's milk started with commercial Lactic Acid Bacteria (LAB) strains following the traditional transformation process, including curd cooking and stretching phase in hot deprotenized whey. Experimental production were contaminated with 10^3 Colony Forming Unit (CFU)/mL of *Escherichia coli* O157, *Listeria monocytogenes*, *Salmonella* Enteritidis and *Staphylococcus aureus*. The experimental plan included three different stretching temperatures: 74.5 °C for 15 min (A), 83.6 °C for 20 min (B), and 90.2 °C for 20 min (C). Microbiological analyses were performed on raw milk, coagulated milk, curds, acidified curds, stretched curds, cheeses after brining and ripened at 30, 60, and 90 days. All samples were subjected to the decimal serial dilution procedure and the microbial suspensions were used for the plate counts of the pro-technological, spoilage and pathogenic populations. Results showed that during milk coagulation, there was a significant ($P<0.05$) increase on the levels of inoculated pathogens followed by a progressive decrease during curd cooking and acidification. After the stretching phase, the loads of added pathogens drastically reduced (<2 Log CFU/g), in trials B and C, and remaining below the detection limit from brining and during ripening. The levels of mesophilic and thermophilic coccus and rod LAB of curds were superimposable among trials and reached values above 6 Log CFU/g in 90-d ripened cheeses. This study evidenced that stretching cheesemaking technology was impactful in limiting the growth of the four selected dairy harmful bacteria, revealing that the best temperature/time combination to be applied during stretching is 83.6 °C/20 min.

P60

How bacteria survive in food-related environments: *L. monocytogenes* and *P. fluorescens* dual-species biofilms in the dairyindustry

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In nature, bacterial biofilms are typically composed of two or more microbial species. For instance, in the dairy industry *Listeria monocytogenes* and *Pseudomonas fluorescens* are often associated in mixed-species biofilms that increase bacterial resistance to disinfectants. Our study evaluated the behaviour of
L. monocytogenes and *P. fluorescens* alone and their interactions in a dual-species biofilm, mimicking the conditions of dairy processing environments. Using a Ricotta-based model system as growth medium, biofilm formation was assessed on stainless steel (SS) surfaces at 12 °C for 7 days. Hence, the biofilm biomass was determined through crystal violet staining, also measuring the sessile cells loads. Moreover, the carbohydrates produced within the extracellular polymeric substances (EPS) were quantified by the anthrone method, and Confocal Laser Scanning Microscopy (CLSM) was used to analyse the biofilm structure. The bacterial species, both alone and in combination, were able to produce biofilm on SS surface. It is noteworthy that *P. fluorescens* increased *L. monocytogenes* sessile load from 1.40 ± 0.20 to 3.58 ± 0.16 Log CFU/cm^2, as well as the total EPS carbohydrates, which reached 2.91 µg/cm² after 72 h. CLSM evidenced the presence of green agglomerates, probably due to the formation of *P. fluorescens* blue pigment. This study highlights the different behaviour of the two bacteria when considered alone and suggests that the interactions between the two species can influence biofilm formation, but not the capability of *P. fluorescens* to produce blue pigment. Given the potential risk for consumers, the stimulating effect of *P. fluorescens* on *L. monocytogenes* adhesion on food contact surfaces should be carefully considered. The results also underline the need to investigate biofilms made of bacterial consortia, which represent the real situationobserved in food environments.

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Microbiome-based tracking of PDO Buffalo Mozzarella geographical origin

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Buffalo Mozzarella (BM) is a typical cheese from Southern Italy with unique flavor profile and texture. It is produced following a detailed Protected Designation of Origin (PDO) regulation, based on a traditional back-slopping fermentation. In this study, we sampled BM from 53 different dairies located in the area of Caserta (n=32) and Salerno (n=21), within the PDO area of production. We assessed Volatile Organic Compounds (VOCs) by gas chromatography-mass spectrometry (GC-MS) and the microbiome by high-throughput shotgun metagenome sequencing. Microbiome taxonomic profiles reveal *Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *delbrueckii*, and *Lactobacillus helveticus* as the dominating microbial species in all samples. However, the differential abundance of taxa resulted in an evident clustering of the samples based on their geographical origin, also showing that BM from Caserta has a greater microbial diversity. Furthermore, we reconstructed Metagenome Assembled Genomes (MAGs) from metagenomes. Different strains of *Lb. delbrueckii* subsp. *delbrueckii* were identified in the samples. In particular, cheeses from Caserta and Salerno area showed different strain profiles. Reconstruction of metabolic pathways related to flavor generation was screened, highlighting different abundance of several microbial pathways according to production area, such as superpathway of 2,3-butanediol biosynthesis and S-adenosyl-L- methionine salvage I. These results may explain the specific flavor profiles of BM from Caserta and Salerno.The microbiome may be regarded part of the terroir that links PDO BM with the specific area of production, also contributing to the peculiar sensorial traits. From this perspective, the metagenomic approach could be helpful in tracking the origin of PDO fermented foods.

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Phages in Trentingrana cheeses' natural whey starters: new insights from characterization and genomic comparison

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Natural whey starters (NWSs) is a quite complex microbial ecosystem traditionally used in the production ofItalian long ripened hard cheeses like Trentingrana, a protected designation of origin (PDO) cheese, typical of the North of Italy and produced starting from raw milk. Daily maintained in the dairy factory from whey collected at the end of the cheese-making process through a back-sloping approach, NWS is characterized by specific niches of thermophilic Lactic Acid Bacteria (LABs) together with the presence of bacteriophages. Deriving mainly from raw milk and dairy implant facilities, bacteriophage infection of NWS's LABs may affect the technological parameters for the cheeses production, representing the most common cause of slow and/or incomplete fermentation.

In this study, we morphologically and genetically characterized seven phages infecting *Lactobacillus helveticus* and isolated from the NWS over one year of Trentingrana production. Six phages were classified as member of Myrovoridae family, while transmission electron microscopy and protein sequences information, allowed to assign one to the Siphoviridae family. All phages harbored gene sets for the typical phage cycle (or injection, replication, assembly and release) and interestingly showing a large set of conserved ORFs, annotated with hypothetical/unknowns function and shared several times between genomes. Providing new insights on the genomic organization of NWS phages, this work underlined the needs of further investigation on the biological role of these sets of potential new proteins, for a better comprehension of their role in NWS-phage maintenance, LABs-phage co-existence, and NWS fitness.

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A multi-omic approach to unravel the adaptation mechanisms of *Listeria monocytogenes* in response to protective cultures in a model system of dairy products

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Listeria monocytogenes is a widespread foodborne pathogen able to grow under environmental stress conditions, such as low temperatures, low pH, and high salt concentrations. The consumption of food contaminated with *L. monocytogenes* causes listeriosis, a disease with a fatality rate of about 20–30 % in older adults or immunocompromised people. The ability to persist in stressful environments makes this bacterium of particular interest for food safety, due to the frequently reported detection in meat and dairy products and raw and processed seafoods, fruits and vegetables. The application of bio-preservation methods using LAB and/or their metabolic products have been extensively studied during the last years to improve safety and quality of foods. Nonetheless, details on the mechanisms of adaptation of *L. monocytogenes* in traditional dairy products during stresses exposure in presence of bio-preservative agents remains largely unclear. In this study, a multi-omic approach was used to evaluate the impact of selected LAB strains over the growth and gene expression of *L. monocytogenes* ATCC-19115 co-cultivated in an experimental cheese- based medium and in a fresh cheese model. Several LAB strains from dairy products and environments were screened for their ability to inhibit *L. monocytogenes* ATCC-19115. LAB strains were furtherly co- cultivated in different conditions with *L. monocytogenes* and those showing the best features to suit the aim of the study were used to perform the challenge test. Growth of *L. monocytogenes* was inhibited in co- cultures assays, both in cheese-based medium and in cheese model. Proteins and genes related to virulence, stress regulation, transcription factors and metabolism were differentially expressed in co-cultivation compared to single culture. This study is expected to provide a deeper insight into *L. monocytogenes* behaviour in presence of natural antagonistic strains, to support the exploitation of bio-preservative strategies to enhance the safety of dairy products.

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Impact of bio-fertilization on sea fennel (*Crithmum maritimum* L.) seed germination, plant growth, and soil biodiversity and health

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Biofertilizers are preparations of living microorganisms that, when applied to seeds or soil, colonize the rhizosphere or the interior of the plant and promotes growth by increasing the supply or availability of primary nutrients to the host plant. The present study was aimed at investigating the impact of biofertilization on seed germination and root growth parameters of sea fennel (*Crithmum maritimum* L.) from two ecotypes under controlled conditions (growth chamber and greenhouse) and demo field. In parallel, the effect of biofertilization on microbial diversity of rhizosphere and bulk soil was also evaluated by viable plate counting and Next Generation Sequencing (NGS) techniques. The biofertilizer was formulated including selected bacterial strain for: i) atmospheric nitrogen fixation (*Azotobacter chroococcum*DSMZ 2286 and *Azospirillum brasilense* DSMZ 1690); ii) phosphorus-dissolving activity (*Priestia megaterium* DSMZ 339); and potassium solubilization (*Niallia circulans* DSMZ 30598). According to preliminary results, biofertilization positively affects seed germination as well as root growth parameters under both growth conditions assayed (growth chamber and greenhouse), whereas no significant differences were seen in the microbial diversity of biofertilized and control bulk and rhizosphere soil.

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P65

The microbiome of *Brassica rapa* fermentation

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Unlike other products, the microbiota of fermented products of plant origin is still rather unexplored. Brovada is a traditional product from Northeast Italy, obtained by the natural fermentation of *Brassica rapa*. In this study we leverage the capabilities of whole genome metagenome sequencing to fully characterize species associated with the progression of fermentation in two different manufacturing plants (A and B), their correlation with relevant physicochemical parameters, and the implications for the functional properties of this product. At the very beginning of the fermentation, Pseudomonadaceae and Moraxellaceae prevailed in both plants. In plant A, Lactobacillaceae were very abundant since the beginning of fermentation, while in plant B they become abundant after 30 days. Plant B showed instead a higher abundance of *Pseudomonas* and *Acetobacter*. At the end of fermentation, the pH was lower in plant A than in B. A strong positive correlation was observed between lactic and acetic acid concentration and prevalence of the *Lactobacillus* genus, while negative correlations were with members of the *Pseudomonas* genus and with *Serratia plymuthica*. Similar correlations were observed for other organic acids, residual sugars, and alcohols. The fungal community at the beginning was also different from those observed during fermentation. The main contributors to this difference appeared to be *Saccharomyces cerevisiae* and several *Pichia* species, present in lower amounts at time 0 compared to subsequent time points. Samples from plant A showed higher levels of five plantaricin genes. Similarly, the number of reads per million mapping to bsh (providing resistance to bile salts) and msa (adherence to epithelial cells) genes were always higher in plant A, which is a strong predictor of probiotic potential. Our results emphasize the possible differences in the microbial populations in different locations, and that such differences can have important health implications.

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Fermented okara: a source of health-promoting polyphenols

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The food production chain generates high levels of waste and by-products all over the world, especially in developed countries. This is the case of okara, the soybean by-product derived from the production of soy milk and tofu. Plant-based foods are known to be rich in bioactive compounds, such as (poly)phenols, which can be catabolized in humans by the host colonic microbiota generating low molecular weight health- promoting catabolites. However, the application of lactic acid fermentation, an eco-friendly approach, to (poly)phenol-rich plant by-products could represent an employable strategy for the valorization of food by- products, since different native compounds may be catabolized, enhancing the quantitative and qualitative profile of bioactive molecules. This study aims to develop and optimize the lactic acid fermentation of okarato obtain a product characterized by a modified bioactive compound profile, possibly enriched with colonic catabolites. To reach this goal different bacterial strains, belonging to the University of Parma Culture Collection (UPCC), were used to explore the fermentative processes and metabolic transformations of the bioactive constituents naturally occurring in okara. To identify the best fermentation protocol leading to the increase of (poly)phenols and the formation of new (poly)phenol microbial catabolites, the Design of Experiment (DoE) was applied. Different factors, both quantitative and qualitative were considered and co- cultures were employed to evaluate a possible synergistic effect. The (poly)phenolic profile of fermented okara in different conditions, was analyzed with ultra-high-performance liquid chromatography (uHPLC) equipped with a mass spectrometer fitted with a heated electrospray ionization probe and was then used to define the optimal fermentation parameters. Overall, during fermentation, the (poly)phenolic profile changed especially for the release of aglycones forms from the glycosylated ones present in unfermented okara.

Different variables were involved in the catabolism of okara during the fermentation process which werehighlighted thanks to the employment of the DoE.

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Lactic acid fermentation of microalgal biomass: a promising source of lipid reducing compounds

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Obesity is one of the most severe threats to human health globally. The search for new bioactive compoundswith lipid-reducing activity is one of the most important hopes to fight this phenomenon. Between several natural resources of bioactive compounds, microorganisms are for sure among the most promising.
Microalgae and cyanobacteria are photosynthetic microorganisms well known for their richness in novel secondary metabolites that exert several bioactivities. At the same time, Lactic Acid Bacteria (LAB) are microorganisms that, thanks to their fermentative metabolism, modify the matrix in which they grow and produce new compounds with potential bioactivities. This study aims to study the production of extracts deriving from fermented microalgae having a lipid-reducing activity and assess it with Zebrafish Nile red fat metabolism assay. *Arthrospira platensis* and two microalgal species (*Chlorella vulgaris* and *Chlorococcum* sp.) biomasses were fermented with seven LAB strains belonging to 4 species (*Lacticaseibacillus casei*, *Lacticaseibacillus rhamnosus*, *Lactobacillus delbrueckii bulgaricus* and *Lacticaseibacillus paracasei*). All the selected strains were able to grow in all the microalgal biomasses and the most suitable substrate for their growth was *Arthrospira platensis*. Microalgae biomass was extracted with methanol, and the extracts were tested at exposures at 10 µg/mL for lipid-reducing activity in zebrafish larvae (*Danio rerio*). *Chlorella vulgaris* and *Chlorococcum* sp. demonstrated a lipid-reducing effect that was not present prior to lactic acid fermentation, while the lipid-reducing capacity of *Arthrospira platensis* biomass was strongly increased by fermentation with *Lactobacillus delbrueckii* subsp. *bulgaricus* 1932. Most promising conditions reduced 70-80 % of neutral lipid reservoirs in zebrafish after 48h exposure. The ongoing analysis aims to identify the responsible compounds by performing metabolite profiling of extracts using HR-ESI-LC-MS/MS and bioinformatics approaches. This work opens the way to new possible sources of lipid-reducing compounds obtained through lactic acid fermentation.

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Multistarter fermentation with selected native strains of *Saccharomyces cerevisiae* and *Schizosaccharomyces japonicus* to valorise the microbial *terroir* of Vignala (TN)

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New trends in winemaking have emphasized the contribution of microbial *terroir* and assessed the use of selected starters from different areas, including non-conventional yeasts or multi-strain starters to diversify the biochemical composition of wines. The aim of this study was to reconstitute the microbial interaction between two native yeasts, previously isolated during a spontaneous fermentation from a historic vineyard in Vignala (Trento, Italy) in 2020, to evaluate the oenological potential of their combination thus valorizing the microbial diversity of the vineyard.
The experimental plan consisted in genotyping the isolates belonging to *Saccharomyces cerevisiae* and *Schizosaccharomyces japonicus*, to identify intraspecific differences. Afterwards, phenotypic characterization assays and analyses of technological characteristics, such as SO_2 tolerance, fermentative vigor and power by impedometric analysis, were performed. The most suitable strains were then inoculated sequentially or in co-inoculum, setting up six different micro-vinifications in must obtained from Vignala vineyard. The different inoculation regimens were compared with performances of a commercial starter in terms of chemical composition of the wines.
Kinetics of the fermentations showed different behaviors, and a yeast dominance analysis confirmed the implantation of the inoculated strains and the dominance of *Sc. japonicus* over *S. cerevisiae* in the mixed culture fermentations. Chemical analyses evidenced remarkable differences in the main oenological parameters and the diversification of wine aromatic profile promoted by the increase of higher alcohols and esters when native strains of *Sc. japonicus* and *S. cerevisiae* were applied as starter cultures.
Based on the results obtained in this study, *Sc. japonicus* shows promising characteristics for wine production due to its contribution in malic acid degradation, glycerol production, and increased concentrations of volatile compounds, making this yeast interesting in terms of increasing the positive attributes of wine.

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Folate production and bifidobacteria: an update

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Natural folates are present in leafy green vegetables, oranges, beans, rice, and liver and many microorganisms, such as bifidobacteria, lactobacilli, and yeasts. Some of these microorganisms are common in the gastrointestinal microbiota and may therefore provide pre-synthesized folate to the host. Folate absorption occurs via transporters in the gastrointestinal epithelia, followed by diffusion through the circulatory system to the liver, where it may be stored in. The human diet varies in folate content and bioavailability, and there is a substantial loss during processing, storage, and cooking. Therefore, the suggested targets for daily folate intake are often uncertain. Certain species and strains of bifidobacteria have shown a strong folate biosynthesis capacity. Gaining a better understanding of which strains are most suitable for it is crucial for fortification programs with bacteria-produced natural folate. The aims of the present work were (i) to describe the current understanding of the distribution of genes needed for complete folate biosynthesis across all bifidobacterial species known today and their hosts; (ii) with a practical appliedfocus, to emphasize the potential for biotechnology and folate-trophic bifidobacteria; (iii) finally, to elaborate on how bifidobacteria (alone or with other microorganisms) may in the future contribute to reducing widespread folate deficiencies prevalent among vulnerable human population groups, such as the elderly, women at child-birth age, and people in low-income countries.

P70

Investigating the transferability of tetracycline resistance in *Listeria monocytogenes* from multi-drug resistant *Enterococcus faecium* in food and environmental settings

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The ability of antimicrobial-resistant (AMR) bacteria to be spread throughout the environment is a major public health concern. This issue has become more relevant especially in the case of the acquisition of AMR in foodborne pathogens that cause a decreased effectiveness of therapeutic treatments.
This investigation aimed to evaluate the transferability of tetracycline resistance, carried by the transposons Tn*916*, from the multi-drug resistant *Enterococcus faecium* UC7251, previously isolated from a fermented dry sausage, to two distinct strains of *Listeria monocytogenes*. The *tet*(M) gene transferability, previously assessed *in vitro* with a conjugation frequency of 10^{-3} transconjugants/recipient, was tested in cheese and meat models, confirming the same rate observed *in vitro*. This phenomenon was also investigated *in vivo*, with three different approaches using *Galleria mellonella* as a screening animal model. The results indicated that the conjugation rate reached frequencies of 10^{-1} by the feeding approach, highlighting the importance ofthe spreading of AMR in the animal gut. Finally, conjugation capacity was tested in a complex marine environment containing *Mytilus galloprovincialis*, as a filtering organism, and polyethylene microplastics as biofilm carriers. *Tet*(M) transfer occurred primarily in mussels, where conjugation rates were 10^{-4}. Furthermore, although at lower conjugation rates, this phenomenon was also observed in salt water. Overall, our investigation showed that both *Listeria* strains were able to acquire *tet*(M) from *Enterococcusfaecium* and that this event occurs in different conditions. *Enterococcus faecium* plays a crucial role in theincrease of AMR because of its intrinsic capacity to acquire and donate genetic material to other bacteria, including pathogens. The monitoring of AMR enterococci should be constantly carried out and further investigations should be implemented to plan mitigation strategies.

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Lactic Acid Bacteria fermentation as a tool toimprove the techno-functional properties of non-wheat flours

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In the last years there has been a growing interest, both by consumers and companies, in using non-conventional flours to produce traditional and novel bakery products, with enhanced texture, flavour and nutritional value. In this work, two different flours have been considered: chickpea flour for its high nutritional profile, and sorghum flour, because is a climate-resilient, gluten-free crop with a high content of bioactive compounds. Despite their positive features, these flours show some limitations: in chickpeas, the presence of anti-nutritional factors and its persistent flavour, whereas for sorghum the major drawback is represented by its protein and starch phases, the former highly hydrophobic which in turns leads poor digestibility of both components. In this context, Lactic Acid Bacteria (LAB) fermentation, could be a valid and sustainable strategy to obtain products with enhanced nutritional quality and improved flavour and texture. The aim of this study was to evaluate the effect of LAB fermentation on the physicochemical properties of chickpea and sorghum batters. For this purpose, the dough was fermented by three LAB strains: *Lactobacillus delbruekii* subsp. *lactis*, *Lacticaseibacillus casei*, *Leuconostoc* sp., selected according to their different phenotypic characteristics and their origin. To assess the growth capacity of bacteria, impedometric analysis, total bacterial count and pH measurements were performed. At the end of fermentation, the water holding capacity (WHC), antioxidant activity (DPPH assay), the proton molecular mobility by 1H NMR and viscosity were evaluated. Our results showed that the tested strains were able to rapidly grow on both flours, reaching concentrations higher than 10^8 CFU/g within 20 hours, and inhibiting most of the contaminants.
This data was confirmed by impedometric analysis, which showed a very short adaptation time (lag) for all the strains. Furthermore, during fermentation, an increase in viscosity was observed probably due to the production of exopolysaccharides by LAB.

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Effects of two freeze-drying procedures on the viability of selected Lactic Acid Bacteria

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Freeze-drying is a widely used preservation technique for microbial culture collections and starter cultures. However, it is a complex process that can damage cells and affect their survival. Among several factors thatcan influence the outcome of the freeze-drying process, pre-freezing temperature can crucially impact the viability and stability of microbial strains. Therefore, optimizing the process is essential. In this study, two freeze-drying procedures were evaluated using five Lactic Acid Bacteria strains from the Unimore Microbial Culture Collection (UMCC, www.umcc.unimore.it). The strains were: *Fructilactobacillus sanfranciscensis* UMCC 2990, *Lactiplantibacillus plantarum* UMCC 2996 *Furfurilactobacillus rossiae* UMCC 3002, *Leuconostoc citreum* UMCC 3011 and *Pediococcus pentosaceus* UMCC 3010. These strains were selected for their reported antibacterial and anti-mould activity and their potential application as freeze-dried starter cultures. Two pre-freezing temperatures were tested: 2 hours at -80 °C and overnight at -20 °C. The viabilityof the lyophilized strains was evaluated, immediately after the procedure, and after seven days of storage at 37 °C to simulate ten years of aging. The results showed that both pre-freezing conditions produced a viability rate greater than 80 % for all strains after the freeze-drying process, and greater than 70 % after the simulated aging storage, except for *F. sanfranciscensis* UMCC 2990, which was the most sensitive strain. Further studies are underway to assessthe effect of the two freeze-drying procedures on strains' fermentative performance, in term of acidification capacity, and on their ability to produce secondary metabolites, comparing the fresh and lyophilized LAB cultures. Overall, this study highlights the importance of optimizing the freeze-drying process for each strainto ensure their viability and stability during long-term storage.

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Co-inoculation of Lactic Acid Bacteria and *Saccharomyces cerevisiae* strains to generate volatile organic compounds with high olfactory impact in Catarratto wine

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The co-inoculation of Lactic Acid Bacteria (LAB) and yeasts ensures time management, completion of malolactic fermentation (MLF) and influences aroma profile of wines. In the present study, three LAB strains (*O. oeni* VP41®, *O. oeni* PN4® and *L. plantarum* MLPK45H®) (Lallemand Inc.) were co-inoculatedwith two *S. cerevisiae* strains (NF213, University of Palermo) collection) and QA23® (Lallemand inc.) to produce Catarratto wine. Length and reliability of MLF differed among trials. The best malic acid degradation kinetics was recorded for the trials with *L. plantarum* strain, MLPK45H® followed by VP41® and finally PN4®.The highest lactic acid yield was displayed by the trial inoculated with *L. plantarum* strain. The co-inoculum of VP41®/NF213 (*O. oeni*/*S. cerevisiae*) strains determined an increase of floral and fruity aromatic components of wines. These results are due to the significant production of 3-ethoxy-1- propanol, ethyl-octanoate and ethyl decanoate. In contrast, the use of PN4®/QA23® (*O. oeni*/*S. cerevisiae*) strains was advantageous in the production of acetate esters such as 3-methyl-1-butanol acetate and phenylethyl acetate compared to control QA23® and all remaining trials. The use of different LAB strains in combination with yeasts was further confirmed as a valid technique capable of reducing the totality of 2,3- butanedione to 2,3-butanediol and 3-hydroxy-2-butanedione to the advantage of the olfactory freshness of wines. Biplot multivariate analysis positively correlated the production of VOCs characterized by low threshold value with MLF length. In conclusion, LAB and *S. cerevisiae* co-inoculation is an important modulator in the production of wine characterized by VOCs with high olfactory impact. Further investigations should be performed to better understand LAB/yeast interaction.

P74

Evaluation of indigenous yeasts associated with sour beer aging asnovel craft brewing starter cultures

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With the increasing success of craft beer, the brewing industry is making efforts to identify new brewer's yeasts suitable for diversifying the sensorial profiles of craft beer. Sour beer is produced by a complex spontaneous fermentation and maturation process. Here, we evaluated the indigenous yeasts inhabiting sour beer during aging as craft brewing cultures. The culturable yeast fraction of natural sour beer consisted of *Saccharomyces cerevisiae* (70 %), followed by *Pichia membranifaciens* (16 %), *Saccharomyces cerevisiae* x *Saccharomyces uvarum* natural hybrids (6 %), *Brettanymces anomalus* (4 %), and *Brettanomyces bruxellensis* (4 %). The pool of yeasts was dereplicated by $(GTG)_5$ REP-PCR genotyping, and a selection of 9 strains (4 *S. cerevisiae*, 1 *S. cerevisiae* x *S. uvarum* hybrid, 1 *B. anomalus* , 1 *B. bruxellensis*, and 2 *P. membranifaciens*) were preliminary screened for maltose consumption. Even though *S. cerevisiae* strains and hybrid WY213 fermented maltose as well as glucose, *P. membranifaciens* WY122 grew on maltose as the only carbon source. *S. cerevisiae* strains were homothallic, moderately sporulated, and produced viable progenies (> 50 %). In hopped wort fermentation (11.5 Plato; 20 °C), *S. cerevisiae* and *S. cerevisiae* x *S. uvarum* hybrid WY213 exhibited the best fermentation rate, the highest fermentation efficiency, and the shortest lag phase; hybrid WY213 produced the highest ethanol content. Both *S. cerevisiae* strains and the hybrid WY213 consumed maltotriose as well as maltose. *P. membranifaciens* WY122 had fermentative aptitude intermediate between *Saccharomyces* and non-*Saccharomyces* candidates. PCA analysis of 53 compounds detected by SPME-GC identified one cluster characterized by acid ethyl esters (with *B. anomalus* WY59, *B. bruxellensis* WY60, and *P. membranifaciens* WY102); the second one by ethanol, 3-methyl-1-butanol, and phenylethyl alcohol (with *Saccharomyces* and *P. membranifaciens* WY122 strains). Globally the present work demonstrated that indigenous yeasts from sour beer could be suitable as brewing starters to confer flavor complexity to craft beer.

P75

Use of *Lactococcus lactis* Q5C6 strain as debitter adjunct culture for cheese clotted with kiwifruit enzymatic extract

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The use of plant-based milk-clotting enzymes in cheesemaking allows to obtain cheeses suitable for vegetarian diet and compliant with the Halal/Kosher standards. The only limitation is given by the high proteolytic activity of these enzymes, which lead to the production of short bitter peptides that alter both cheese flavor and texture. However, by using specific bacterial aminopeptidases (aminopeptidases N and X) is possible to further hydrolyze these short peptides, reducing bitter taste in cheese. In the present work the debittering activity of a *Lactococcus lactis* (Q5C6) strain was investigated: the latter was used as adjunct culture in cheese clotted with kiwifruit enzymatic extract to improve the sensory profile of the final product. In detail, the optimal amount of kiwifruit enzymatic extract, to be used in cheesemaking, was determined by performing laboratory-scale coagulation tests. Furthermore, two experimental cheesemaking trials (A and B) were carried out and the obtained cheeses were subjected to physico-chemical, microbiological, and sensory analysis. Results of laboratory-scale coagulation tests show that the use of 0.7 g/L of kiwifruit enzymatic extract allowed to obtain acceptable coagulation time and slight bitter taste. Data revealed that the use of the kiwifruit enzymatic extract determined changes in fat, ash, and protein content in the final cheeses compared to cheese clotted through the animal rennet. Moreover, the *Lactococcus lactis* Q5C6 strain, used as adjunct culture at 1 % (v/v), decreased the bitter taste, generating a cheese with a sensory profile comparable to cheese clotted by animal rennet. In conclusion, *Lactococcus lactis* Q5C6 is a promising strain in cheese debittering, produced with kiwifruit clotting enzyme.

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P76

Selection of bacterial cellulose-producing Acetic Acid Bacteria fromkombucha tea and evaluation of the influence of culture conditionson bacterial cellulose production

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Bacterial cellulose (BC) from Acetic Acid Bacteria is widely investigated as biopolymer for different applications. The interest in BC is due to its high-water holding capacity, good mechanical strength, elasticity and high crystallinity. Among Acetic Acid Bacteria, *Komagataeibacter xylinus* plays the most important role because it is considered the highest producer of BC. However, the BC production can bestrongly affected by several variables.

In this context, the aim of this work was to perform an initial screening among 10 strains belonging to *Komagataeibacter* sp. and *Novacetimonas* sp., previously isolated from kombucha samples in order to select the strain able to produce the highest amount of BC. Then, the effects of different culture parameters (pH, Temperature, sources of carbon and nitrogen) were evaluated on the selected strain and specifically, ontheir capacity to produce BC in static cultivation.

Among the tested strains, *Komagataeibacter* sp. showed the highest yield of BC production during static fermentation in medium containing glucose or mannitol as carbon source. Regarding the effects of culture conditions, the results showed that all the parameters tested affected the yield of BC production. Among the carbon sources, mannitol, glucose and fructose allowed to obtain the highest yield of BC. Regarding nitrogen source, yeast extract gave the highest yield. The pH (4–6) resulted suitable for BC production. The temperature of 25-30 °C resulted optimal for BC production with the higher yield obtained at 30 °C.

The results of this study provide further insight into the role of strain selection, medium composition and physico-chemical fermentation parameter control in the improvement of BC production by acetic acid bacteria during static fermentation.

P77

Identification of stress-induced proteomic changes in a virulent*Listeria monocytogenes* strain

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Adaptive stress response is a key factor for the survival of *Listeria monocytogenes* in foods and food environments. This study aims to investigate the immunogenic proteins (IP) encoded by *L. monocytogenes* ST7 following exposure to environmental stressors. To give a better understanding of the role of the proteinsexpressed under stress conditions, *L. monocytogenes* cells were cultivated at 37 °C, pH 7.0, NaCl 0.5 % – optimal condition (C1) – and 12 °C, pH 5.5, NaCl 7 % (C2), and were collected at late exponential growth phase. The proteins were extracted by enzymatic reagents, precipitated, solubilized, and then quantified by BCA method. Proteins identification was performed by shot-gun nLC-MS/MS approach, filtering and selecting only those proteins identified with at least 2 peptides against *L. monocytogenes* Uniprot database. The IP prediction was obtained by mean of sublocalization and immunogenic software. Gene enrichment analysis was performed by STRING v11.05.
To face stress environmental conditions, *L. monocytogenes* encoded for proteins associated to cell wall (lmo1090, lmo1091, lmo0933, lmo2550, lmo0497), which might have an impact on host-pathogen interaction as Internalin A (InlA) and Inhibitor apoptosis (Iap) proteins, well known virulence factors involved in host cell adhesion.
Moreover, these factors were absent in optimal condition (C1), in which instead a sugar uptake cluster (lmo0097, lmo0098, lmo0781, lmo0782, lmo0099, fruA) was identified. This cluster was positively regulated by Sigma B (σB) virulence and Accessory gene regulator (Agr), which are associated to quorum sensing system and cell survival. Our findings suggest that these proteins may be involved in the adaptation of *L. monocytogenes* to environmental stress factors and may be important to explain the general stress response and the pathogenesis of *L. monocytogenes*.

P78

Unravelling the impact of sourdough fermentation on rye phytochemicals in a whole-grain dough system

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The use of sourdough fermentation is increasingly being re-examined as a sustainable bioprocessing technology to improve functional and nutritional features of baked goods. While huge progress has been made in elucidating the ecology of sourdough microbiomes, less is known about how whole grain phytochemicals are affected by microbial metabolism and food processing.
The aim of this study was to evaluate the impact of selected, sourdough-related Lactic Acid Bacteria (LAB) fermentation on the chemical composition of rye flour in combination with seed sprouting. Whole-grain rye doughs were prepared with a sourdough starter including *Limosilactobacillus fermentum*, *Weissella confusa* and *Weissella cibaria*. Metabolic profile was assessed using ultra-high performance liquid chromatography coupled to a quadrupole time-of-flight mass spectrometer. High performance anion exchange chromatography with coupled pulsed amperometric detection was used to determine carbohydrates pattern. Sourdough fermentation resulted in a decrease of monosaccharides and low-polymerization degree (PD)-oligosaccharides, which was considerably higher than that observed in doughs fermented with yeast only.
VIP analysis revealed an increase in the mannitol content of rye doughs when LAB were added as starters, suggesting an interesting implication for sugar-reduced product applications. Concurrently, LAB metabolismpromoted a general up-regulation of the dough system metabolome, leading to the accumulation of several bioactive molecules, including terpenoids, flavonoids, phenolic acids like p-coumaroyl derivatives, stilbenes, coumarins and lignans. This group includes well-known precursors of antioxidant and aromatic compounds, which may potentially contribute to enhance health benefits and organoleptic properties of derived breads.
Among lipid metabolites, doughs fermented with sourdough LAB displayed a significantly higher amount of lysophospholipids that can extend the shelf-life of baked goods by slowing down the staling process.
Overall, these results shed light on LAB fermentation-induced modifications of content, properties and biological availability of rye grain constituents, providing insight to guide the development of new healthyand nutritious rye-based products.

P79

Comparison of metabarcoding taxonomic markers to describe fungal communities occurring during natural Falanghina wine fermentation

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Falanghina is a top product of the Sannio (Campania Region, Italy) terroirs. Next generation sequencing offers several ways to study microbial communities. For vine/wine sciences, identifying species in diverse areas is the key for understanding the role of microorganisms both during grape production and during winemaking, and to improve quality and sustainability. Choosing the most robust barcode marker for accurate description of fungal communities is crucial. The aim of this study was to compare different markergenes for metataxonomic studies of fungal diversity in wine ecosystems. Three targets (ITS1, ITS2 and D1/D2 regions of rDNA) were tested for profiling fungal communities. Six samples of grapes, coming from different terroirs and relative musts during natural fermentation were analyzed. Mycobiota was mainly represented by Ascomycota, showing an abundance ranging from 91.3 - 4.4 % (by ITS2) to 98.2 - 2.8 % (by 26S). However, the three different approaches showed a very different number of fungal genera: 63 by ITS2,20 by ITS1 and 17 by 26S. Moreover, only in few cases the taxonomic resolution reached the species level. The core mycobiota as resulted by ITS2 is represented by 18 different genera with a different distribution depending on the sample origin (terroir) and the stage of fermentation. The most occurring yeast genera (covering at least the 70 % of population) were *Metschnikowia, Hanseniaspora*, *Saccharomyces*, *Pichia*, *Aureobasidium* and *Starmerella*. However, using a culture-based approach, we also found isolates identified as *Candida*, *Wickerhamomyces*, *Rhodotorula* and *Sarocladium*.
In conclusion, although ITS2 appears as the most accurate marker for must and wine samples the taxonomicresolution within some genera may be not enough to reach a species-level identification. This further highlights the need to combine metabarcoding with culture-dependent analyses.

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P80

Use of *Lachancea thermotolerans* and *Saccharomyces cerevisiae* isolated from manna ash products to improve quality of loquat beer

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Manna ash products are obtained from several species of *Fraxinus* sp. During summer season, the trunk and the main branches of the tree are notched by incision with cutters and phloem sap, a cerulean liquid, is collected. On contact with the air, this liquid quickly thickens and forms a light crystalline whitish layer that represents manna. The high sugar content (about 80 % w/w) makes this matrix an extremely selective raw material for microorganisms with potential food applications. Among isolates, the most abundant microbial group was represented by yeasts and, in particular: *Lachancea thermotolerans*, *Saccharomyces cerevisiae*, *Citeromyces matritensis*, *Starmerella* sp., *Candida* sp. and *Zygosaccharomyces* sp. In recent years, there is an increasing interest in generating beers brewed with the inoculum of non-conventional yeasts and novel raw material and/or ingredients such as fruits. In this study, a technological selection of yeasts to produce craft beer with the addition of loquat fruits has been performed. Commercial yeast strains of *S. cerevisiae* and *L. thermotolerans* were used as controls, while *S. cerevisiae* MN113 and *L. thermotolerans* MNF105 were used as selected strains. At the end of alcoholic fermentation, an amount of loquat juice was added to the must up to 20 % (v/v) of total volume. Results of sensory analysis showed that sour fruit beers produced with *L. thermotolerans* MNF105 were more sensory balanced (acidity versus sweetness) with intensity of flavours higher than control beer. These results are imputable to a low lactic acid production by the selected strains. The overall organoleptic investigation showed a preference for experimental beer produced with *S. cerevisiae* MN113. Aldehydes and alcohols were the most abundant compounds emitted from the beers produced. In conclusion, manna-related yeasts *S. cerevisiae* MN113 and *L. thermotolerans* MNF105 represent promising starters for the production of fruit beer and light sour fruit beer.

P81

Non-conventional yeasts (*Starmerella lactis-condensi* and *Candidaoleophila*) and Lactic Acid Bacteria (*Lactiplantibacillus plantarum* and *Oenococcus oeni*) in sequentially inoculated fermentations: a strategy to improve aroma of Catarratto wine

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Catarratto is one of the most common non-aromatic white grape varieties cultivated in Sicily (Southern Italy). To improve the aromatic expression of Catarratto wines, different combinations of non- *Saccharomyces* strains and Lactic Acid Bacteria (LAB) were applied during experimental vinification. Two non-*Saccharomyces* strains, (*Starmerella lactis-condensi* MN412 and *Candida oleophila* YS209) isolated from "manna" and two commercial strains of lactic acid bacteria (*Lactiplantibacillus plantarum* MLPrime™ and *Oenococcus oeni* VP41™) were used in sequentially inoculated fermentations. The strain *Saccharomyces cerevisiae* (QA23™) was inoculated to complete the alcoholic fermentation. Control trials were conducted without the inoculum of non-*Saccharomyces* and/or LAB strains. The experimental design resulted in nine different treatments (S1-S9). Microbiological counts showed the ability of *St. lactis-condensi*and *C. oleophila* to persist at high cell densities (6.0 Log CFU/mL and 5.5 Log CFU/mL, respectively) up to six days of fermentation. *L. plantarum* and *O. oeni* performed malolactic fermentation in the inoculated trials(with levels above 7.0 and 8.0 Log CFU/mL respectively). The dominance of the two non-*Saccharomyces* strains over native yeast populations was estimated to be more than 90 % as revealed by genotypic strain typing. In terms of chemical parameters, *St. lactis-condensi* increased glycerol content by about 2-3 g/L and *C. oleophila* showed lower acetic acid content than the other trials. The use of *St. lactis-condensi*or *C. oleophila* increased the aromatic complexity of the wines as reflected by volatile organic compounds (VOCs) composition and sensory profiles. Forty-two VOCs were identified, and they were mainly represented by esters (ethyl acetate, ethyl octanoate and ethyl lactate), alcohols (1-pentanol and 2,3- Butanediol), aldehydes, ketones and carboxylic acids. Results of sensory analysis showed a significantincrease of floreal/fruity intensity and complexity of Catarratto wines produced with *C. oleophila* and *St. lactis-condensi* in combination with *L. plantarum*.

P82

Survivability of an encapsulated commercial probiotic strain in theupper gastrointestinal tract through a SHIME model

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The oral administration of probiotics under fasting or feeding conditions is not entirely clear. The aim of the present work was to detect the survivability of the commercial probiotic strain *Lacticaseibacillus rhamnosus* CA15 (DSM 33960) in the upper GI tract, under fasting and feeding conditions, using an *in vitro* human gastrointestinal microbial ecosystem (SHIME) model. A double-jacketed reactor was used to sequentially simulate the upper GI digestion, maintaining a temperature of 37 °C at constant agitation. Under fasted condition, the stomach was simulated with a 45 minutes incubation in a gastric juice (pH 2.0), while under fed condition the stomach incubation was simulated over 120 minutes using a modified gastric juice (pH 4.5) containing the SHIME® nutritional medium (ProDigest, Ghent, Belgium). Both conditions were subjected to duodenal (27 min from pH 2.0 to 6.5), jejunal (63 min from pH 6.5 to 7.5), and ileal (90 min, pH 7.5) fractions, previously supplemented with pancreatic juice. Samples were collected from both stomach and small intestine fractions and subjected to plate cultivation using MRS medium incubated under anaerobiosis at 37 °C for 48 hours.

Under fasting condition, the CA15 probiotic strain was not detected in the stomach portion, probably due to the viable but nonculturable state, whereas a cell density of 6.73 Log CFU/mL in duodenum, followed by 7.03 Log CFU/mL in the jejunum, and 6.83 Log CFU/mL in the ileum was detected. However, under fed condition, a cell density of 6.27 Log CFU/mL was recovered from the stomach fraction. The same trend was observed in both duodenal (6.63 Log CFU/mL) and jejunal (6.63 Log CFU/mL) fractions with a slight decrease in the ileal portion (5.97 Log CFU/mL). In conclusion, the CA15 strain showed good survivability under both tested conditions (fasted and fed), suggesting its suitability to be administrated under the stressfulfasted conditions.

P83

Sustainable approaches to mould control on Provolone Valpadanacheese

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Plasticoat with natamycin added is the main method used to control the shelf-life of pasta filata cheeses, such as Provolone Valpadana PDO. Natamycin is an antibiotic used at a concentration of 1-10 mg/kg and is considered an additive. Since the consumer's tendency is to prefer a clean label, the aim of the research was to identify new packaging to create a more sustainable and natural product. In particular, two types of coatings were evaluated: one consisting of gelatine and chitosan, the second formulated with gelatine supplemented with LAB, *Weissella confusa* and *Weissella cibaria*. Chitosan is a naturally occurring, edible and biodegradable polymer. The two *Weissella* strains were capable of producing exopolysaccharides with a protective function, showing also antifungal activity. The first part of the research involved *in vitro* testing of the two coatings. Challenge test was performed using two moulds isolated from pasta filata cheese: *Penicillium roqueforti* and *Aspergillus candidum*. The best results were obtained with the coating composed of gelatine and chitosan, which was able to reduce significantly *P. roqueforti* growth and to inhibit *A. candidum*. Subsequently the research was applied on entire 1 kg wheel of Provolone Valpadana PDO by coating it with gelatine and chitosan and fungal inoculation. The cheese samples were cured at 16 °C, at room humidity for 1 month. The chitosan-coated Provolone did not show any fungal development as Provolone control with natamycin-supplemented plasticoat. In conclusion, the coating proposing the combination of chitosan and gelatine represents an excellent alternative to the use of natamycin for the control of fungal development in pasta filata cheeses. Chitosan-based coatings provide an interesting change of technology for the dairy industry and consumer, as they are sustainable, biodegradable, edible and easy to apply.

P84

Microbiota characterization of Italian traditional artisan cheeses: anew certification level

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Dairy products can be considered a complex ecosystem influenced by multiple factors. In the production of traditional cheeses, the microbial community is conditioned by raw milk, starter cultures and environment. Italian traditional cheeses are known for their unique flavour profiles that can be attributed to the microbiota that develops during ripening. The present study aims to characterize, through a metataxonomic approach, the microbial communities in traditional dairy products and Protected Designation of Origin (PDO) cheeses to set up a database on microbial composition for food safety purposes and to promote traditional products via microbial biodiversity. Dairy products were included in the study if made of raw milk, ripened for short period (60-day maximum), produced without commercial microbial starters.
Samples of raw milk, curd and cheese at two different ripening times were collected in two dairy factories located in Piedmont, NorthWest Italy, in summer and fall 2022. About all milk samples analyzed, regardlessof origin, a majority of *Pseudomonas*, *Acinetobacter* and *Chryseobacterium* are observed. For the curd samples (DairyA), the most represented genera are *Streptococcus*, *Acinetobacter* and *Pseudomonas* and in DairyB *Acinetobacter*, *Enterobacter* and *Flavobacterium*. Related to cheeses, in the final product of DairyA the genera *Streptococcus*, *Leuconostoc* and *Psychrobacter* are mostly observed in the summer production; in the fall production, the *Psychrobacte*r is replaced by *Enterobacter*. The cheese from DairyB reports the most represented genera as *Lactococcus* and *Serratia*, and in the fall production the emergence of *Enterobacter* is also observed. The microbial communities varied between the two different kinds of cheese, suggesting that production methods and environmental conditions could contribute to the development of characteristic microbiota. Overall, our study could provide valuable insights into the microbial communities characterizingtraditional Italian cheeses.

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P85

Selection of *Saccharomyces* yeasts for the production of polyphenol- enriched wine

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Consumer interest in the close correlation between nutrition and health has helped promote intense research into bioactive compounds in foods. The polyphenols in wine, in addition to being important in determining its organoleptic characteristics, are considered to be primarily responsible for the beneficial effects this beverage promotes on human health. The concentration of polyphenolic substances in grape must is high, but during winemaking, their total content is substantially reduced (by more than 50 percent) by adsorption onto yeast wall polymers and subsequent lees separation. This issue can be addressed by using *Saccharomyces cerevisiae* strains with a lower tendency to adsorb polyphenolic compounds. The *S. cerevisiae* strains under study were identified from those contained in the CNR ISPA microbial collection. Specifically, 136 different strains were evaluated by laboratory-scale fermentation analysis. The results obtained showed that all strains decreased the concentration of polyphenolic compounds and, consequently, the antioxidant power. The best strains, i.e., those that differed in their lower ability to adsorb polyphenols, were selected and characterized during the various phases of the study. By processing the data obtained at the end of the different experimental phases, we were able to identify four strains, based on their qualitative performance. In fact, using the aforementioned strains, the wines obtained are found to have a high concentration of total polyphenols and an implemented antioxidant capacity, hold optimal aromatic properties, and have very low levels of volatile acidity and undesirable metabolites. Taken together, the scientific results described above emphasize the relevance of the development and industrial application of innovative biotechnological approaches in order to enhance the presence of healthful molecules in wine, thus improving "functional parameters" resulting in improved quality of the final wine.

P86

An integrated approach to explore the microbial biodiversity ofnatural milk cultures for cheese production

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Natural starter cultures from whey (NWC) or milk (NMC) have always been considered a fundamental element of the quality and distinctive features of many PDO and STG cheeses, for which their use is mandatory. The complex microbial ecosystems of NWC and NMC are the result of heat treatment and incubation conditions, which can vary considerably among different production plants. While much is known about WNC microbial communities, data on NMCs are still scarce and limited to the use of culture- dependent techniques, which are not able to fully capture the microbial biodiversity of these complex matrices. In this study, a targeted 16S-sequencing approach coupled with a physicochemical characterization was used to explore the microbial biodiversity of NMCs for Montasio, an Italian PDO cheese. A predominance of *St. thermophilus* was detected, followed by Kurthia, which suggested the use of milk that has undergone prolonged refrigeration. Specific sporeformers were also detected, probably due to their survival to thermal treatment. A low prevalence (around 0.1 %) of lactobacilli (*Lactobacillus helveticus*, *L. delbrueckii*, and *L. salivarius*) was detected. Diversity indices were negatively correlated with incubation temperature, and pH increased as biodiversity indices increased. The incubation temperature would therefore be a main driver of biodiversity in NMCs. Also, heat treatment temperature resulted negatively correlated, albeit not significantly, with diversity. On the contrary, a weak positive correlation was observed for the time of heat treatment and incubation. The volatilome of NMC showed the presence of 35 compounds, including acids, alcohols, ketones, aldehydes, and sulfur compounds. According to the PCA biplot and hierarchical analysis, NMC samples were grouped into four leading clusters. We can assume that this microflora, characterized by distinct metabolic pathways, would be able to exert them also inside the cheese, affecting its sensory profile.

P87

Antimicrobial resistance, pathotypization and clonal relatedness of cloacal *Escherichia coli* from healthy lamb differently fed

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In livestock environment, healthy animals can constitute a reservoir of potentially dangerous *Escherichia coli* that can cause asymptomatic infections in animals and that can pass through the food chain causing disease in humans. Our objectives were to assess both the presence and abundance of *E. coli* in healthy Sicilian lambs, differently fed, to study their virulence profile, their resistance to antimicrobials and to evaluate the effects of diet on the *E. coli* presence. Two hundred *E. coli* isolates were characterized by PCR for virulence genes, for antimicrobial resistance and clustered through PFGE. No differences in abundance and distribution of *E. coli* strains among control and treated groups were revealed. Shiga toxin-producing E. coli (STEC) were most frequently isolated, with the prevalent presence of the stx1 gene, while the enterotoxigenic (ETEC) strains were occasionally observed, and the genes defining enteropathogenic (EPEC), enteroinvasive (EIEC) and enteroaggregative (EAEC) *E. coli* were never detected. Phenotypic antibiotic susceptibility profiles showed remarkable differences among isolates. Overall, a high prevalent against tetracycline was detected in both control and treated groups, while differences for colistin, ciprofloxacin, and trimethoprim/sulfamethoxazole, were pointed out especially in control group. 84.5 % of *E. coli* strains exhibited a multidrug resistance. The clonal relatedness of isolates, explored through by XbaI- PFGE, showed high genetic diversity among the strains, highlighting the dominance of the unique pulso- types. In conclusion, the high prevalence of multidrug-resistant *E. coli* in healthy animals, together with the abundance of several pathotypes highlighted the importance of healthy lambs as *reservoirs* for the dissemination of the antibiotic resistance determinants to humans. However, data suggested a promising efficacy of the diet on virulence traits.

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P88

Microbial diversity and dynamics of Cosacavaddu cheese from differently fed cows

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The biodiversity of traditional cheeses significantly contributes to the sensorial properties of the fina product, on shelf life, and on the overall cheese quality.
The aim of the present study was to evaluate the effects of based by-product diet, as cow dietary supplementation, on microbiota of Cosacavaddu cheeses, a brine-salted pasta filata raw-milk cheese, aged for 3 to 12 months. The cheeses are produced in the Hyblean region of Sicily from raw milk using traditional wood tools without any commercial starters. Cheeses from conventionally fed cows, were used as control. In detail, two cheesemaking trials were conducted, and cheese samples at different days of production and (from 0 to 150 days) ripening were subjected to plate counting and PCR-DGGE (denaturing gradient gel electrophoresis) analyses to investigate the structure of the bacterial community and its distribution during ripening. Microbiological data showed the absence of *Salmonella* sp., *L. monocytogenes*, *E. coli*, and fecal coliforms in all cheese samples. Lactic Acid Bacteria (LAB) trend was quite similar in both control and experimental cheeses. The highest cell density was achieved in experimental samples (7.97 CFU/g) at 30 days of ripening, which was quite constant till 60 days and slightly decreased till 120 days. Both mesophilic and thermophilic lactococci showed similar trend in both samples. PCR-DGGE fingerprinting revealed slight microbial dynamics differences among control and experimental samples, probably due to the effect of the higher polyphenol compounds. This study highlighted that the resilient core microbiota Cosacavaddu cheeses and its dynamics throughout ripening were not affected by the feeding strategy, contributing to the sustainability use of food by-products.

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P89

Use of autochthonous LAB and yeast starters for the production of whole-wheat bread

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Sourdough is one of the oldest starters traditionally used for making baked goods, offering several advantages to the sensory, rheology, and shelf life of final products. The present study aimed to formulate a LAB and yeast starter culture to be used as sourdough for the production of the whole-wheat bread. Strains ascribed to *Levilactobacillus brevis*, *Lactiplantibacillus plantarum*, and *Saccharomyces cerevisiae* species, previously isolated from traditional Sicilian sourdough samples, were mixed in order to setup white and whole-wheat flour experimental sourdoughs. The ratio between LABs and yeasts was monitored during the fermentation by plate count. In addition, rheological properties as well as pH, Total Titratable Acidity (TTA), and Dough Yield (DY) were evaluated. Experimental white and whole-wheat breads, manufactured in a local bakery using a mature dough (one daily back slopping for 21 days), were subjected to physico- chemical analysis.
Microbiological analysis showed a constant LAB/yeast ratio of 100:1 through the fermentation process. A constant decrease of the pH was revealed in all the experimental sourdough samples, reaching a mean value of 3.7 after 48 hours of fermentation. White wheat flour sourdough samples showed higher TTA than whole-wheat sourdoughs. Based on the calculated DY, all samples were categorized as doughy. A different physico- chemical profile among white and whole-wheat bread samples was revealed. In particular, higher content of calcium, iron, total fatty substances, omega 6 fatty acids, polyunsaturated fatty acids, and carbohydrate was detected in white wheat bread samples than whole-wheat breads, which showed high protein, fiber, and ash content. In conclusion, the autochthonous LAB and yeast isolates, used in the present study, revealed promising technological properties and can be successfully applied in whole-wheat bread production.

P90

Evaluation of heat treatment parameters to ensure microbiological safety and nutraceutical value of donkey's milk

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The objectives of this study were to evaluate the microbiological quality of donkey milk (DM) heat treated in different farms and to determine the effects of heat treatment on nutraceutical properties of Sicilian DM, under laboratory scale.
Milk samples were routinely collected from 4 farms right after heat treatment, carried out at different conditions (from 69 to 72 °C for 30 s – 2 min). Moreover, raw milk was sampled eight times at monthly intervals. The latest samples were treated at 63, 72, and 76 °C for 15 s, 2 min, 10 min, and 30 min, and analyzed for Lysozyme Activity (LA), using a Sigma® kit; Total Antioxidant Capacity (TAC), measured through the Bioquochem® e-BQC NI device. In addition, the Alkaline Phosphatase Activity (ALP), according to ISO 11816-1, was detected. Zooming on microbiological results *Listeria*, *Salmonella*, *Escherichia coli* O157 and *Campylobacter* were never detected after any heat treatments applied at farms.
However, coagulase-positive staphylococci showed count values lower than 2.00 Log CFU/mL, with some exceptions. The detected TAC value in raw DM was 568 e-BQC/mL and decreased by 14-23 %, independently of the applied treatment. Raw DM showed a LA of 8263 U/mL and a LA loss up to 5 and 20 % were estimated after 15 s at 72 °C and 76 °C treatments, respectively, confirming that treatments at temperatures higher than 72 °C should be avoided. Similar LA losses were observed when treatment was performed at 72 °C for longer than 15 s. Regarding ALP after treatments, results highlighted values always lower than 350 mU/L, even at sub-pasteurization conditions, underlining that the ALP could not be used as index of pasteurization, as food-safety regulations stated.

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P91

Potential of Lactic Acid Bacteria strains as bio-preservative agents inminimally processed orange cloves

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Minimally processed fruits are ready-to-eat products that maintain their freshness and nutritional quality if properly treated. A major problem of fresh-cut fruits is their limited shelf-life. An alternative strategy to avoid microbial growth and to improve the shelf-life is the use of Lactic Acid Bacteria (LAB) and their metabolites as bio-preservative agents. To this purpose, in the present study LAB strains were isolated from the endocarp of Tarocco oranges. The strains were phenotypically and biochemically characterized by conventional tests (microscope observation, catalase test, Gram stain) and by API 50 CHL Medium kit.
Then, the strains were tested for ability to grow in stressing conditions, such as at low pH (pH 3.0, 4.0 and 4.5), and temperatures (8 ± 1 °C and 4 ± 1 °C). Additionally, the potential antibacterial effects of the strains against pathogens commonly found in minimally processed fruit, as *Escherichia coli*, *Pseudomonas aeruginosa*, *Listeria innocua* and *Staphylococcus aureus*, were evaluated. The phenotypic and biochemical tests identified the isolates as belonging to *Lactiplantibacillus plantarum* species, as confirmed by molecular profile using species-specific PCR. They showed a high tolerance to pH 4.5, reaching a final concentration of 10^9 CFU/mL. In relation to cold conditions, two strains (AS1 and AS3) showed a good tolerance at 4 ± 1 °C, reaching a final concentration of 10^8 CFU/mL. The antimicrobial activity of cell-free supernatants (CFS) of the selected strains was *in vitro* tested using the disc diffusion method. Based on the obtained results, the CFS was applied as bio-preservative solution into minimally processed orange slices, packaged in passive atmosphere and samples analyzed during refrigerated storage. Microbiological results revealed a spoilage agents reduction during the shelf-life of orange slices.

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Microbiome dynamics and antibiotic resistance gene patterns alongbeef food chain

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The food chain has been recognized as one of the key routes of transmission of antibiotic resistance (AR) from animal to human bacterial populations. The increasing numbers of reports on the presence of antibiotic resistance in food bacteria are indicative of an important public health problem globally and, consequently, there is an urgent need to limit the spread of antibiotic resistance genes (ARGs) within food chains, since they may be transferred to opportunistic and pathogenic bacteria.
To effectively mitigate or counteract the AR problem, identification and characterization of ARGs as well astheir transmission routes and mechanisms of action is crucial.
The ongoing development of shotgun metagenomic approaches is providing the means to explore AR infood microbiome.
For this purpose, reads and metagenome assemblies coming from a cattle processing industry were screened by shotgun metagenome sequencing, to investigate the distribution patterns of the microbiome and ARGs, since this chain may represent a *reservoir* of ARG-carrying microorganisms and a hotspot for the transmission of ARGs.
Retail shop's samples were more similar among them and showed a similar resistome composition, clustering apart from the slaughterhouse surfaces, that also showed higher microbial diversity.
In particular, carcass hosted a broader range of ARGs, mainly attributed to Aminoglycoside, Tetracycline and Folate pathway antagonist classes while the proportion of AR contigs for slaughterhouse and retail shop area turns to be lower. Furthermore, carcass, slaughterhouse and retail shop hosted antibiotic resistance genes mainly attributed to *Acinetobacter* and *Psychrobacter*.
Our findings highlight the correlations between the bacterial community and the resistome, providing acomprehensive overview of the ARGs *reservoir*.

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Reuse of almond by-products: scale-up production of functionalalmond skin added semolina sourdough breads

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Almond by-products are rich in polyphenols. A previous work carried out at laboratory-scale level demonstrated the functionalization of traditional semolina sourdough bread with almond skin, but also evidenced some hygienic issues related to the storage condition of this almond by-product. The present work reports the application of powdered almond skin (PAS) for sourdough bread production at industrial level.
To this purpose, almond factory generating wet almond peel modified the producing line to ameliorate the hygienic characteristics of the skin to be reused. Plant modifications reduced the presence of spore-forming aerobic bacteria, members of Enterobacteriaceae family and total coliforms below the detection limits.
Starting from three recipes without (control, CTR) or with PAS (at 5 or 10 % (w/w) on the weight of semolina, 5-PAS and 10-PAS, respectively), seven different sourdough breads were realized: ciambellina, galletto, sfilatino, lunette, pagnottella, mafalda, and chiocciolina. Sourdough inoculum (pH 3.77) was characterized by a fermentation quotient of 2.68. The initial pH of control trial was 5.16, while higher values were registered for PAS added doughs. The higher titratable acidity registered in PAS added doughs was mainly imputable to the acid content of almond by-products. After fermentation, LAB slightly increased for all doughs, while the increase of yeasts was more pronounced due to the addition of baker's yeast. After baking, PAS addition determined a lower weight loss, an increase of firmness and a diminution of specific volume. A preference test indicated that mafalda and sfilatino shapes were those more appreciated by consumers and further investigated. Mafalda breads formulated with 10 % PAS, after 6-day storage, exhibited the highest IC50 value of 90.77 mg/mL against DPPH radical, while a moderate ABTS radical scavenging activity was observed among investigated samples. Sensory evaluation is ongoing.

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P94

Production of functional vinegar and non-alcoholic fermented beverages from olive mill wastewater: a biotechnological approach

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Olive mill wastewater (OMWW) is a by-product resulting from olive oil extraction. It is a dark liquid, still rich in polyphenols, assimilable carbon, and lipid residues, that is a serious ecological issue for agri-food industry because of its highly pollution and plentiful output. Unfortunately, in some geographical areas, OMWW are also illegally disposed of soil and waterways, causing incalculable environment damage.
Therefore, finding a way for an alternative use of OMWW is a huge challenge for scientific community and food industry. The common goal is reducing the environmental impact and having an income for being able to tempt the reuse of this source of active compounds. The aim of this study was to value OMWW as suitable matrix to obtain functional vinegar and non-alcoholic fermented beverages. Firstly, the total phenolic content was determined according to Folin-Ciocalteu's method and HPLC analysis was used to identify and quantify several organic acids, sugars, and polyphenols. Then, OMWW were filtered, centrifuged and microfiltered to remove solid particulate, clarify and sterilized. Subsequentially, because the chemical composition of OMWW could be a limiting factor for microbial growth, alcoholic fermentation trials by *Saccharomyces cerevisiae* (UMCC 855) were carried out using 100 %, 75 % and 50 % (v/v) of OMWW added with of sucrose at 10 % (w/v). The alcoholic product using 50 % (v/v) of OMWW was shown to yield the highest ethanol content, so it was subjected to acetic fermentation by *Acetobacter pasteurianus* (UMCC 1754). A final fermented beverage containing functional phenols was obtained and characterized.
This study provides a suitable biotechnological process by which the fermentative potential of OMWW could be exploited producing new functional vinegar and non-alcoholic fermented beverages.

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Taxonomic characterization of halotolerant bacteria from traditional Italian cheeses

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Halotolerant microorganisms have current or potential applications in various biotechnological fields, also providing new solutions for the production of fermented food. The dairy processing environment particularlyrepresents a *reservoir* of halotolerant lactic and non-lactic microorganisms with unexplored properties.
Following the evaluation of their safety-in-use, these bacteria can be used as adjunct starter cultures on the rind of cheeses, to enhance their sensory characteristics, improve safety profile, and positively modify the surface cheese microbiota. These pro-technological microbial populations still appear little investigated in Italy, and not at all applied during dairy processing. It is therefore of interest the characterization of the halotolerant microbiota of dairy products. To this aim, dairy samples, including brine, milk, curd, cheese at different ripening times, rennet, whey starter, and processing environment, were collected from the production process of "Caciocavallo Pugliese" and "Salva Cremasco PDO" cheese. Halotolerant lactic and non-lactic bacteria were isolated using specific culture media, biotyped, and subsequently identified through16S rRNA sequencing.
In the case of Caciocavallo Pugliese, the cell density of halotolerant bacteria during the production process ranged from 2.00 to 5.00 Log CFU/g, whereas they reached the maximum cell density of 6.00 Log CFU/g in theSalva Cremasco cheese and 8.50 Log CFU/cm^2 in the environmental swabs. More than 300 strains were isolated in total. Large part of the genera identified were included in the phyla Proteobacteria, Bacillota, Firmicutes, and, to a lesser extent, Pseudomonadota, Actinobacteria, and Actinomycetota. Among the isolated species, *Marinilactibacillus psychrotolerans*, *Carnobacteriummobile*, *Leuconostoc mesenteroides*, *Hafniaalvei*, *Bacillus halotolerans* could potentially be used as surface adjunct starters. The physiological and biochemical characterization of these strains and their application as adjunct cultures in a model cheesesystem will be the next steps in this work.

P96

Microbiological and chemical-physical composition of kombucha inrelation to starter cultures

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Kombucha is a trending beverage obtained by the fermentation of tea by a complex microflora containing yeasts and Acetic Acid Bacteria. Kombucha can be a valid low-calorie substitute of soft drinks as it is a sour, naturally carbonated and sweet tasting drink. However, despite the increased interest, the microflora and functional properties of kombucha have not yet been fully understood. The aim of this work was to characterize from a microbiological, chemical-physical, functional and sensorial point of view three types ofkombucha obtained by fermenting green tea containing sugar with different starter cultures. Through metagenomic analysis it was possible to identify 14 genera and 34 microbial species. However, yeasts, and in particular *Brettanomyces bruxellensis*, resulted the dominant kombucha microflora. However, the different types of kombucha had differences in terms of Acetic Acid Bacteria (*Komagataeibacter* sp., *Acetobacter* sp., *Gluconobacter* sp., *Gluconoacetobacter* sp.) and yeasts (*Starmerella* sp., *Schizosaccharomyces* sp. and *Zygosaccharomyces* sp.). Ethanol and acetic acid were the dominant molecules of the volatile profiles of kombucha, however, the samples differed from each other for the content in alcohols, esters and acids. All the samples showed a high antioxidant potential linked to the high content in phenols. This study confirmed the good sensory and functional properties of kombucha and indicated that the microflora responsible for the fermentation process can significantly affect the characteristics of the final product.

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16S rRNA metabarcoding survey and cultivable approach reveal novel insights on microbial interactions in natural whey starter forParmigiano Reggiano cheese

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Parmigiano Reggiano cheese-making process entails the usage of natural whey starter (NWS), a complex microbial community produced from whey of the previous cheese-making round, by application of high temperature gradient. Selective pressure acting during this incubation shapes the structure and composition of undefined mixtures of several strains and/or species of Lactic Acid Bacteria present in NWS. Even if NWS is critical to assure consistent and reliable downstream cheese-making steps, little is known about the composition, functional features, and plant-to-plant fluctuations. This study encompassed the collection of 10 NWS samples across PDO area of Parmigiano Reggiano cheese to investigate the microbiota by culture-dependent methods and 16S rRNA metabarcoding. 16S rRNA metabarcoding analysis revealed two main NWS community types, namely NWS type-H and NWS type-D, with *Lactobacillus helveticus* being more abundant in NWS type-H and *Lactobacillus delbrueckii* / *Streptococcus thermophilus* in NWS type-D, respectively. Prediction of metagenome functions indicated that NWS type-H samples have enriched functional pathways related to galactose catabolism and purine metabolism, while NWS type-D were enriched in pathways related to aromatic and branched chain amino acid biosynthesis, which are flavor compound precursors. Culture-dependent approaches revealed low cultivability of individual colonies as axenic cultures and high genetic diversity in the pool of cultivable survivors. Co-culturing experiments showed that fermentative performance increases by increasing the microbial complexity of synthetic community, suggesting that complex biotic interactions and cross-feeding relationships take place in NWS and assure phenotypic robustness. This preliminary study provides the basis for experiments aimed at understanding how selective regime and raw milk quality affect NWS composition and microbial interactions, as well as its technological features.

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Parmigiano Reggiano cheese as *reservoir* of bioactive peptides producer Lactic Acid Bacteria

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Functional food can exert a key role in prevention and mitigation of chronic degenerative diseases that are increasing in Western population, such as type 2 diabetes, hypertension, and atherosclerosis. Milk and milk- derived food and beverages promote human health and wellness due to their content in bioactive molecules and probiotic cells. Most of these bioactive molecules come from metabolic activities of Lactic Acid Bacteria (LAB). LAB can hydrolyze milk caseins releasing bioactive peptides (BP) with anti-hypertensive, anti- diabetic, and antioxidant functions. Here we screened a pool of 39 LAB strains isolated from Parmigiano Reggiano cheese for their capability to release caseins-derived BP during milk fermentation. Preliminary, proteolysis degree was evaluated after 72 h of fermentation. The most proteolytic candidates, namely *Lacticasebacillus rhamnosus* RBH10 and *Lacticasebacillus zeae* CBH02, CBR01, and CBK04 were further tested in milk fermentation trials over time to monitor the evolution of acidification, microbial count, proteolysis degree, and biological activities of Low-Molecular Weight (LMW) peptide fractions. *Lcb. zeae* CBK04 and *Lcb. rhamnosus* RBH10 overcame the other candidates in producing fermented milk with the highest angiotensin-converting enzyme (ACE) and dipeptidyl peptidase IV (DPP-IV) inhibitory activities.
UHLPC/HR-MS analysis defined the peptide profiles of LMW peptide fractions collected after 72 h of fermentation and revealed that *Lcb. zeae* CBK04 and *Lcb. rhamnosus* RBH10 released the lactotripeptides Valine-Proline-Proline (VPP) and Isoleucine-Proline-Proline (IPP), which exert antihypertensive activities in in vitro and in vivo trials. Globally, the present study demonstrated that Parmigiano Reggiano cheese is a *reservoir* of mesophilic LAB strains suitable as GRAS cell factories to produce milk-based functional beverages enriched in bioactive peptides.

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Back to the future. Making an innovative starter for kefir production starting from a traditional kefir grain

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Kefir is a fermented drink traditionally produced from a natural starter, the kefir grain, which is characterized by a complex microbiota. At an industrial level, kefir is generally made starting from selectedstarter cultures in freeze-dried form, providing a better preservation compared to the natural grains inoculum. Starting from their own traditionally used kefir grain (kefiran), the company Bionova, producing kefir and starter cultures, looked at the future, investigating the possibility to create a microbial starter resembling their original kefiran.
On one hand, the work envisaged the isolation of colonies on selective culture media and the genetic identification of the microorganisms detected, while in parallel a metataxonomic analysis of the kefir grain was carried out, with the aim of observing the relative abundances of the species present in it.
A total of n° 52 colonies of different morphology have been isolated from plates, both for bacteria and yeasts population. Following DNA extraction, bacteria species were subjected to genotyping rep-PCR and electrophoresis, with the aim of identifying clusters of genetically related microorganisms. From each cluster a single species was then selected and sent for 16S rDNA sequencing. A total of 13 bacterial isolates were sequenced. However, the sequencing results showed, the presence of only 4 bacterial species. The metataxonomic analysis of the grain, detected 5 different bacterial species. The same was done for yeasts, with 15 colonies isolated, which were finally identified as one single species. Among the bacterial species observed, only two were found to be common to both approaches, thus highlighting that the combination of the two methods can better highlight the complexity of undefined microbial starters.
This work was the starting point to set up a new starter culture to potentially increase the standardization ofkefir production while maintaining the peculiar characteristics of the original kefir grain.

P100

Study of the effects of pasteurization and of selected microbial starters on functional traits of fermented table olives

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Table olives are one of the most known vegetable fermented foods, being a fundamental component of the Mediterranean diet. Their production and consumption continue to increase globally and represent an important economic source for the producing countries. One of the most pivotal challenges in the next future is the modernization of the olive fermentation process. Beside the demand for more reproducible and safer production methods able to reduce products losses and potential risks, producers and consumers are increasingly interested in the health characteristics of the final product.

In this study, the use of microbial starters from different sources were tested for both their technologicalfeatures and their potential ability to improve functional traits of fermented black table olives.

The contribution of microbial starters to the table olives debittering process was fully described in terms of specific enzymatic profile, microbiological profile, nutrient components, fermentation-derived compoundsand content of bioactives.

For each fermentation assay, the effect of controlled temperature (constant at 20 °C) and uncontrolled (ambient) temperature as well as the consequences of the pasteurization treatment were tested on the finalproducts. Starter-driven fermentation strategies seemed to increase both total phenolic content and total antioxidant activity.

Herein, we provide data indicating that, among all combinations tested in this study, two bacterial strains (*Leuconostoc mesenteroides* KT 5-1 and *Lactiplantibacillus plantarum* BC T3-35), and two yeast strains (*Saccharomyces cerevisiae* LI 180-7 and *Debaryomyces hansenii* A15-44) were the most performing ones from the technological and functional point of views. We demonstrated that the best choice for producing black table olives was to ferment the raw material under uncontrolled temperature conditions and that pasteurization can have a role in enhancing the levels of the antioxidant compounds.

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Bio-protective cultures to control the spoilage of perishable softcheeses due to undesired bacterial proliferation

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Soft-fresh cheeses such as Crescenza and Mozzarella are characterized by high moisture content and availability of several nutrients that can easily allow proliferation of contaminating bacteria during their short shelf-life. In this work, challenge tests were carried out to evaluate the possible inhibition of target spoilage bacteria by means of two bioprotective cultures belonging to *Lactococcus lactis* (Bio1 and Bio2). In Crescenza cheese, a pool of coliforms and Leuconostocs able to grow even at refrigeration temperatures, which may cause early gas production with visible bloated food packages, were used as target spoilage bacteria. Contaminants were inoculated in pasteurized vat milk (< 100 CFU/mL) and the protective cultures were added as adjunct to the primary starter culture. Cheeses were analyzed at the end of fermentation and weekly until 28 days from production. Both Bio1 and Bio2 did not exert control during the cheesemaking stages but significantly reduced the growth of coliforms and Leuconostocs of about two orders of magnitude during shelf-life compared to control (only starter culture and contaminants). In citric Mozzarella cheese (produced without starter culture), the target contaminants were psychrotrophic strains of *Pseudomonas* able to produce blue pigments and responsible of blue discoloration defect during storage. A pool of "blue" *Pseudomonas* sp. and the two bioprotective cultures were both inoculated into the brine solution used as preservation liquid for storage of packaged citric Mozzarella cheese. Samples were monitored weekly during 30 days of shelf-life. In this case, the protective culture Bio1 was ineffective whereas the culture Bio2 significantly reduced the proliferation of spoiling *Pseudomonas*, limiting the likelihood of the occurrence of blue discoloration defects. Overall, both for Crescenza and citric Mozzarella cheeses, the use of the protective culture Bio2 showed a progressive sensory impact (softening of the texture) throughout the shelf-life.

P102

Yeasts ecology and volatiles profile of spontaneously fermentedTaggiasca black and green table olives

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Taggiasca table olives are produced with a spontaneous fermentation carried out by placing the raw drupes directly into a brine with a salt concentration of 8-12 %. Such concentrations favor the growth of yeasts, and this process can last up to 8 months. During this period, yeasts are mainly involved in the debittering of the fruits and the production of volatile compounds. In this study, the microbial ecology of naturally fermented Taggiasca table olives was evaluated through culture-dependent and independent methods coupled with volatilome analysis via GC-MS. Two fermentation batches were considered and both brines and olives samples were analyzed. At the end of the process, yeasts populations reached 3.8 and 6 Log CFU/mL in olives and brines, respectively. Culture-dependent analysis revealed that the most abundant species overall were *Candida diddiensae, Wickerhamomyces anomalus, Pichia membranifaciens* and *Aureobasidium pullulans*, with many differences in species distribution between batch 1 and 2. Culture-independent analysis confirmed the presence and dynamics of *W. anomalus* in batch 1 in brines and olives throughout the entire fermentation, whereas *Cyteromyces nyonsensis* and *Aureobasidium* sp. dominated the fermentation of brines and olives in batch 2, respectively. Volatilome results were analyzed and correlated to the microbiota data, confirming differences between the two batches. Such variations in microbiota and volatiles profile contributed to a successful fermentation of batch 1 and a poor fermentative process in batch 2, which did notproceed to the packaging step. This study will help improve the knowledge on the main microbial groups of the Taggiasca variety and their relationship with the quality of the final product. It will also guide the selection of potential autochthonous starters to better control the fermentative process while preserving the sensory qualities of the product.

P103

Metabolic profiling microarrays for targeted sustainable processes

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Microorganisms are the main representatives of biodiversity in all ecological habitats. Among them, Lactic Acid Bacteria (LAB) include a wide range of genera and play important roles in several food industries. Over the time, LAB evolved the ability to metabolize different carbon sources which allow them to colonize various niches. Food waste and by-products represent alternative substrates to produce industrially interesting biochemicals. The fermentation of these matrices provides significant economic benefits and encourages sustainable development promoting a circular economy.
This work aims to investigate the metabolic functionalities of a selected microbial core (SMC) from a wide collection of LAB, by exploring their biodiversity, with the final outlook of carrying out targeted fermentation processes by recovering food waste and by-products.
The SMC consists of 150 strains of LAB of food origin belonging to the University of Parma Culture Collection (UPCC). Strains were genotypically characterized by Amplified Fragment Length Polymorphism(AFLP), which provides a subspecies-level resolution fingerprint. Substrates utilization patterns of strains were determined on 71 different carbon sources by using BIOLOG GenIII MicroPlates. To describe the biodiversity and the metabolic functional diversity of the SMC, ecological diversity indices were calculated. Genotypic results show an overall evenness of clusters as LAB belonging to the same species mainly fall into the same group. On the other hand, the phenotypic microarrays highlight substantial differences among substrates assimilation profiles also within the same species. It remarks that the metabolic biodiversity is greater than that traditionally associated with the source of isolation. Moreover, the most frequently consumed C sources characterize the composition of various food waste and by-products. This suggests that a focused selection of strains can be exploited to build a microbial platform for valorization of food leftovers and develop targeted sustainable processes.

P104

Emmer flour sourdough development and effect of fermentation onnutritional features

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Emmer wheat is a hulled species, whose cultivation was replaced in the 20th century by modern wheat varieties, owing to its low yield and productivity. Nevertheless, the growing interest in natural and organic products has led to emmer rediscovery, thanks to its nutritional properties and adaptability to low-input and organic farming system. As regard to bread-making, generally long fermentation processes, such as sourdough, are considered more suitable for emmer flour processing. Thus, the aim of this work was to develop an emmer flour sourdough and evaluate its stability during time. Several nutritional and chemical features (free and total phenolic compounds, protein profile by SDS PAGE, and antioxidant capacity) of 4 emmer varieties (named Vitreo, Mucronato, Produttivo and Precoce) as well as the effect of the microbial fermentation on these characteristics were assessed. Sourdough development was monitored by determining the pH, the total titratable acidity, and microbial concentrations, until these parameters were stable. The identification of the occurring microbiota indicated the presence of two Lactic Acid Bacteria (LAB) species, *Companilactobacillus paralimentarius* and *Lactiplantibacillus plantarum*, and a yeast species, *Saccharomyces cerevisiae*. Vitreo variety exhibited the highest total phenolic content (TPC) (1402 mg/L) byFolin-Ciocalteu assay, the highest concentration of free phenolic compounds (3711 mg/L), particularly syringic, ferulic and sinapic acids. Each variety was used to prepare doughs fermented by axenic or mixed cultures of *S. cerevisiae* and LAB, isolated from the emmer sourdough. Dough with lower pH exhibited higher TPC and antioxidant capacity. The type of fermentation, rather than the emmer variety, affected the free phenolic compounds profile, that were similar among samples with the same inoculum, especially concerning the p-coumaric, ferulic, protocatechuic, and syringic acids concentrations. In conclusion, the research led to the isolation of autochthonous LAB and yeasts well-adapted to emmer flour, able to increase emmer nutritional characteristics

Poster Session 2

HUMAN microbiota as a tool for a sustainable future

P105

Evaluation of bile salt hydrolase activity of promising probioticstrains with potential cholesterol-lowering activity

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Bacterial cholesterol-lowering activity is counted among probiotic characteristics, and it is mainly related to bile salt hydrolase (BSH) mechanism. BSH is explicated by gastro-intestinal bacteria, which can reduce cholesterol absorption in intestinal lumen, with a consequently decrease of cholesterol blood levels. The aim of the present study was to evaluate the ability of *Lactobacillus* and *Bifidobacterium* strains to deconjugate BSs, to formulate functional foods with hypocholesterolemic effect. In this study, 66 potential probiotic bacteria, isolated both from food and human origins, have been investigated for their ability to deconjugated taurodeoxycholic acid (TDCA) and glycodeoxycholic acids (GDCA). The test has been performed through a direct qualitative plate assay, using modified MRS supplemented with BHs. An overnight culture of each strain has been inoculated into MRS-TDCA and MRS-GDCA plates, and a bile acid precipitate around the colonies was observed, indicating BSH deconjugation.
Thirty of the tested strains showed a positive reaction against the two BSs. In detail, 24 strains have resultedpositive against TDCA, 4 strains against GDCA, and only 2 strains have deconjugated both TDCA and GDCA. The deconjugation of bile salt hydrolase, was revealed by the presence of an opaque halo around theinoculated strains or by the formation of opaque granular white colonies.
In conclusion the present study demonstrated that the qualitative plate assay could be considered a good preliminary *in vitro* test to evaluate the BSH activity of bacterial strains, in order to select strains with promising cholesterol reduction activity.

P106

Meta-omics approach as a tool to investigate patients suffering fromlow-grade inflammatory pathologies

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Based on the crucial role exerted by complex microbial communities on human health in intestinal niches and, considering the microbiota impact in related pathologies, a coordinated and massive effort is required tocollect and analyse samples from stratified and diversified patient cohorts.
Delving the microbiota involvement in low-grade inflammatory pathologies, we retrieved our samples from the first Italian biobank (based on the BIOMIS project) collecting saliva, stools, vaginal derivatives, blood, and serum. The biobank includes various noncommunicable diseases (NCDs) and from this whole set we picked fecal samples relative to low-grade inflammatory pathologies i.e., chronic kidney disease, type 1 and type 2 diabetes together with healthy control subjects. The samples were then used for metabolomics and metaproteomics analyses.
Better detailing, metabolomics profiles were obtained by using a gas chromatograph coupled with a massspectrometer and analysed with the NIST library.
High-resolution metaproteomics sample spectra produced with an Orbitrap mass spectrometer were processed through the usage of the dedicated bioinformatics Trans-Proteomic Pipeline, TPP version 6.2.0 developed by the Seattle Proteome center and run on server Linux server provided with an Apache web server. Within the configured tools, starting from spectral data, the Comet software modules (https://comet- ms.sourceforge.net/), granted the relative quantification of peptides and proteins by comparing the resulted hits against an ad hoc customized sequence database.
Python scripts have been used to extract protein entries and to create a non-redundant (https://github.com/pwilmart/fasta_utilities) UniProt database (Swiss-Prot + TrEMBL) provided together with its decoy section, useful for FDR computing. Omics data in sample groups were compared and onlystatistically significant hits were kept.
The evaluation of metabolomics and metaproteomics results allows for depicting a complete framework of possible markers made of metabolite and protein panels in low-grade inflammatory pathologies.

P107

Development and characterization of fermented soy beverages containing encapsulated or non-encapsulated vaginal probiotics

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Human microbial niches such as the healthy vagina have recently been discovered as "unconventional" sources of probiotic candidates that can prevent various vaginal diseases. These microorganisms could be offered as oral preparations, as they can reach the vaginal niche via the gastrointestinal tract. However, their use in food would be challenging. The aim of this work was to develop and characterize (from a technological, biological, and nutritional point of view) fermented soy beverages, containing encapsulated and non-encapsulated vaginal lactobacilli (*Lactobacillus crispatus* BC4 and *Lactobacillus gasseri* BC9), as possible nutritional strategies for vaginal dysbiosis. Viability of the vaginal strains remained stable throughout 28 days of storage at 7 log CFU/mL of product, despite the use of encapsulated or non- encapsulated bacteria. Samples with encapsulated bacteria, especially E-BC4+BC9, showed higher water- holding capacity, higher lactic acid content and remarkable antagonistic activity against enteropathogens. In addition, encapsulation protected the strains from simulated GIT conditions, but reduced the acceptability of the final products. Overall, strains BC4 and BC9, alone or in mixture, proved to be promising co-starter cultures, imparting a characteristic flavour (pleasant odour and taste) and aroma (less hexanal, benzaldehyde and more diacetyl and 2,3-pentanedione compared to the control) to the fermented soy beverages. Moreover,products containing vaginal strains had higher nutritional value (higher levels of protein, α-linolenic acid, iron, magnesium, vitamin B2 and amino acids), a lower lipid quality indices but a higher in vitro protein bioaccessibility. Eventually, the digested products, obtained upon simulated stomach and duodenal incubation, were tested on a simulated post-menopausal human gut microbiome and showed a donor dependent effect on the microbial population and metabolism.

P108

Human milk microbiota: correlations with fatty acids compositionand diet in the first six months after delivery

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Human milk is considered an ideal example of a natural functional food containing nutrients, hormones, growth factors, immunoglobulins, cytokines and enzymes that alone meet the needs of infants up to 6 months of age. Besides this, several studies have shown that human milk contains different communities of bacteria, letting infants consume from 10^5 to 10^7 cells of bacteria/day. According to the most recent hypothesis, the human milk microbiome would originate from a translocation of bacteria from the mother's gastrointestinal tract to the mammary gland through an entero-mammary pathway. Therefore, maternal nutrient intake, which is thought to directly affect the maternal gastrointestinal bacterial community, may also indirectly affect the milk microbiota. In addition, several associations between human milk fatty acid profiles and variations in milk microbiota have been documented. Monounsaturated fatty acids in milk werenegatively associated with Proteobacteria but positively associated with the genus *Lactobacillus*. Polyunsaturated fatty acids (PUFAs) may have a dose-dependent inhibitory effect on bacteria, including *Lactobacillus*, while lauric acid, *cis*-9/*trans*-11 and *trans*-10/*cis*-12 conjugated linoleic acid may inhibit *in vitro* the growth of *Staphylococcus aureus*. These scientific findings seem to confirm a physiological link with the overall composition of the milk microbiota and fatty acid concentration, which, as already mentioned, is also closely linked to maternal nutrition. Therefore, the aim of this study was to perform an integrative analysis of the milk microbiota and fatty acids content, considering important factors (e.g., maternal diet, delivery mode, maternal BMI and other lifestyle variables) influencing these human milk components during the first six months after child delivery.

P109

Enhancing the digestibility of Pinsa Romana: an investigation usinga simulated static *in vitro* model

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Manufacturing parameters and fermentation conditions interfere with the nutrients content of baked goods and affect their gastrointestinal fate. Pinsa Romana is a type of pizza that, recently, has been commercially rediscovered but that needed elucidation from a nutritional and digestibility perspective. To cover this gap, we have characterized for the first time six types of Pinsa Romana (five made with indirect method and one produced with straight dough technology) for their biochemical and nutritional features. Several variables like indirect (biga) Pinsa Romana production process, fermentation time and inclusion of sourdough were investigated. The Pinsa Romana produced with biga including sourdough led to the lowest predicted glycemic index (pGI), the highest levels of overall peptides, free amino acids, and gamma-amino butyric acid (GABA), as well as to the best protein quality indexes. The digesta from a static *in vitro* digestion simulation confirmed a reduced *in vitro* glycemic response after intake and highlighted a lower bioavailability of hydrophilic peptides. Moreover, the inclusion of sourdough in biga during manufacturing improved the bioavailability of protein related end-products including human health promoting compoundssuch as essential amino acids and peptides.

P110

Differences of the gut microbiota and metabolome in patients affected by different types of nephropathies: chronic kidney disease, diabetic kidney disease, autosomal dominant polycystickidney disease, and immunoglobulin a nephropathy

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The impaired kidney function in nephropathic patients was linked to an unbalanced microbiota asset. To better detail the reasons of this dysbiosis, we here inspected the gut microbiota and volatile metabolome in four kidney pathologies: Chronic Kidney Disease (CKD), Diabetic Kidney Disease (DKD), Autosomal Dominant Polycystic Kidney Disease (ADPKD), and Immunoglobulin A nephropathy (IgAn).
This observational study led to determining differences between the four groups, compared to a healthy control group (HC). Biochemical parameters and faecal samples were used to investigate the gut microbial population through an approach based on quantitative PCR and gas chromatography coupled with mass spectrometry.
Blood biochemical variables highlighted four distinct profiles relative to the four groups of nephropathic patients. Looking at qPCR results, a discriminant analysis of principal components led to the separation of the above mentioned microbial profiles from HC and ADPKD with respect to CKD, DKD, and IgAn groups, that appeared to cluster together. Most impacting loading factors included *Bifidobacterium*, *Lactobacillus*, *Bacteroides*, and *Clostridium* species and *Bifidobacterium*, *Prevotella*, *Desulfovibrio* and *Atopobium* at the genus level. On the other hand, based on the volatile metabolic analysis, the investigated groups were plottedin three clusters. IgAn was characterized by greater amounts of esters and ketones, ADPK and CKD by aldehydes, terpenes, sulfuric compounds, and fatty acids, while DKD and HC by greater concentration of indoles, hydrocarbons, alcohols, phenols, and carboxylic acids.
All the investigated nephropathy led to an altered homeostasis. By comparing the different kidney relatedpathologies, we detected some volatile organic metabolites and biochemical parameters that resemble differentiated host symptomatic conditions. In the presence of an impaired kidney function, these results shed light on the way the gut microbiota is shaped by different clinical pictures and on how some metabolites and taxa can be used as markers useful to predict and stratify patient groups.

P111

Characterization of the gut microbiota and metabolome in healthyand unhealthy obese patients

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Metabolically healthy (MHO) and unhealthy (OB) obese patients showed different risk of related comorbidities, e.g., diabetes, cardio-metabolic diseases, and cancer. The gut microbiota plays a pivotal role in the regulation of host metabolism and several studies reported the link between obesity and dysbiosis. Here, based on clinical data and dietary life-style habits, the characterization of gut microbiota and faecalvolatile metabolome were investigated in both MHO and OB cohorts.
We here carried out an observational study where obese patients stratified into two groups were compared with healthy control subjects (HC). Blood samples and food questionnaires were collected. Faecal samples were used for characterization of i) gut microbiota, through quantitative PCR; and ii) gut metabolome with gas chromatography coupled with mass spectrometry. A dedicated statistical approach, i.e., rotating factor and discriminant analysis of principal components, was used to focus the attention on a subpanel of variable that clinically support the pathologic obese status.
Biochemical profile of HC and MHO subjects showed statistically significant differences compared to OB patients. Taxa content in terms of presence/absence and abundances was inspected and, as a result, the abundance of *Clostridium coccoides* was higher in HC subjects than both obese groups. *Lactobacillus* genusand *Lactiplantibacillus plantarum* were detected to be higher in MHO subjects compared to HC, whereas a greater amount of *Prevotella*, *Desulfovibrio*, and *Lactiplantibacillus plantarum* characterized OB subjects. Pattern of metabolites, including acetone, beta-myrcene, alpha-terpineol, and other volatile compounds, differentiated the three groups. In addition, Butanoic acid was lower in OB subjects, compared to MHO. The gut microbiota differences between healthy and obese subjects contribute to the understanding of the association between the gut microbiota and these metabolic diseases and, if confirmed by further large-scale studies, could represent potential biomarkers for a less invasive diagnosis of morbid obesity.

P112

In vivo evaluation of an innovative synbiotics on stage IIIb-IV chronic kidney disease patients

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Microbiota unbalance has been proven to affect chronic kidney disease (CKD) patients, and both microbiota composition and activity are implicated in CKD worsening. The kidney failure progression implies an exceeding accumulation of nitrogenous-derived waste compounds in both blood circulating system and intestinal milieu. Therefore, in the presence of an altered intestinal permeability and reduced kidney-based clearance, gut-derived uremic toxins – mainly indoxyl sulfate (IS) and p-cresyl sulfate (PCS) – accumulated. In a scenario facing the nutritional management as an adjuvant therapy, the present study investigated the effectiveness of an innovative synbiotics for its ability to modulate the patient gut microbiota and metabolome by setting a randomized, single-blind, placebo-controlled, pilot trial and enrolling IIIb-IV stage CKD patients and healthy controls. The innovative synbiotics comprised a mixture of *Bifidobacterium animalis* BLC1 (10^9 cells), *Lacticaseibacillus casei* LC4P1 (10^9 cells), fructo-oligosaccharides (2.5 g), inulin (2.5 g), quercetin (640 mg), resveratrol (230 mg) and proanthocyanidins (13 mg). Fecal metataxonomicsand volatilome were analyzed at the run-in, after two months of treatment, and after one month of wash out. Based on multivariate approaches, in CKD patients that were allocated in the synbiotics arm, the top-5 metataxonomics variables reaching significance accounted for Firmicutes, Lachnospiraceae and *Blautia* increase, while Bacteroidetes and Flavobacteriaceae decrease. Noteworthy, a carry-over effect till the end of the wash out was observed. In the same stool samples, acetic and propanoic acids, as well as 2-tridecanone and decane, increased while dimethyl trisulfide and nonanoic acid showed the opposite. In conclusion, the here analyzed data emphasized a selective efficacy of the innovative synbiotics on stage IIIb-IV CKD patients also considering the beneficial effects observed for some CKD-associated clinical parameters (i.e., reduced free circulating IS, improved small intestine barrier integrity, and ameliorated abdominal pain and constipation syndromes). Nonetheless, a further validation based on an increased patient number should be considered.

Poster Session 3

ENVIROMENT microbiota as a tool for a sustainable future

P113

The sunflower rizosphere alters the floral VOCs profile influencingthe pollinators visits

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Honeybees can provide important ecosystem services for society through pollination of crops. Some crop productions are rich in nectar, resulting in a benefit for both honeybees and beekeepers. However, in the last decade, new varieties of crops have been selected for commercial purposes without evaluating the impact on the attractiveness to pollinators in terms of nectar production or VOCs profile. For example, sunflower has been genetically selected for oil production with high oleic and linoleic acid content but has recorded a drop in nectar secretions. The inability of a genotype to establish an appropriate and beneficial "dialogue" with soil microorganisms, responsible for the solubilization of microelements useful for nectar and VOCs emission, could be one of the main causes in the decline of this crop for honey production.
To shed light on these aspects, a field trial involving a high oleic hybrid sunflower variety (LST907) and a non-hybrid variety (Peredovick) was established in 2021 and 2022. both sunflowers varieties were divided into plots, and the rhizosphere was inoculated with different microbial inoculants of *Bacillus*, *Paenibacillus*, *Pseudomonas*, *Lactobacillus*. Flowers were collected from the different experimental conditions and immediately stored in vials in dry ice for VOC profile analyses (SPME-GS). At the same time, samples of the rhizosphere were collected and the microbial community was analyzed in NGS. The results showed that the colonization of the rhizosphere by selected microorganisms depends on the sunflower variety.
Furthermore, the inoculation of some microbial genera did not result in effective colonization of the rhizosphere but rather in an essential alteration of the rhizosphere microbial profile compared to the control. Finally, the microbial treatments in the rhizosphere significantly altered the VOCs profile. These results are a first step in the understanding of soil's biological fertility, with respect to plant growth and pollinators' attractiveness.

P114

Assessing the influence of bioplastics on microbial activity duringthe organic waste treatment and discovering strategies for overcoming accumulation in compost

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Bioplastics were introduced as potential environmentally friendly alternatives to conventional fossil-based plastics. The typical valorization treatments for the Organic Fraction of Municipal Solid Waste (OFMSW) emerged as possible end-of-life options also for post-consumer bioplastics. However, their impact on the microbial communities involved in this treatment remains largely unexplored. Two studies were conducted to investigate the anaerobic digestion and aerobic composting of OFMSW mixed with bioplastics, namely Poly (Lactic Acid) (PLA) and Starch-Based Bioplastic (SBB). These experiments were designed to replicate real industrial conditions of time and temperature. Both laboratory-scale and pilot-scale experiments were conducted to assess the biodegradability of materials, and the quality of the final composts, and to study microbial communities throughout the process. To evaluate the impact of PLA and SBB on bacterial, archaeal, and fungal diversities, High-Throughput Sequencing (HTS) technology was employed at various stages of the process. The results from the experiments yielded distinct outcomes. At laboratory-scale, the bacterial and fungal populations exhibited notable biodiversity differences in aerobic biofilms formations and compost derived from PLA, while the archaeal community showed fewer dissimilarities. On the contrary, at pilot-scale, the presence of bioplastics had a more pronounced effect during the aerobic composting stage. Further studies are necessary to gain a deeper understanding of the effects of these rapidlyexpanding bioplastics on the microbial structures involved in OFMSW treatment and explore the potential relationship between microorganisms and bioplastics.
In parallel, to accelerate the biodegradation processes of any bioplastic residues, strains with higher biodegradation abilities were identified from a collection of microorganisms isolated from bioplastic waste. Comprehensive genomic analyses were conducted to gain a thorough understanding of the genetic pathways responsible for the degradation of bioplastics and selected microbial strains were assembled into customized microbial *consortia* with enhanced degradation capabilities to be employed in bioaugmentation tests.

P115

Influence of diet and host genotype on the bacterial community of *Drosophila melanogaster*

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Insect gut symbionts are involved in important processes related to host development, nutrition and physiology. Factors such as diet are known to contribute to the gut microbiota modulation; conversely, host genotype effect has been less explored. Here, we investigated the gut bacterial community associated with the larvae of the model species *Drosophila melanogaster* with the aim to evaluate their structure and abundance as a function of genotype and diet factors. Considering genotypes, Pngl loss-of-function mutant and the respective not-diseased heterozygous strains were considered: *Pngl* mutation causes a congenital disorder of deglycosylation, resulting in the reduction of the insect life span, gut malformations and sever midgut clearance defect. Standard laboratory diet or high-fat diet were administered to the fly genotypes. The structure of the gut bacterial community was analysed by high-throughput 16S rRNA gene sequencing: larvae of both genotypes were inhabited by *Lactobacillus* and *Acetobacter*, being dominated by *Lactobacillus*.
PCoA analysis showed a differentiation of the gut bacterial communities according to the diet. Bacterial abundance, evaluated by qPCR, was statistically higher in guts dissected from homozygous larvae than heterozygous ones, being coherent with the food-retention phenotype showed by homozygous larvae. A culture-based approach confirmed this result. Thus, while there are no differences between the genotypes of
D. melanogaster with regards to the composition of the gut bacterial community, there was a significant difference when considering the bacterial abundance. Food substrates were also investigated showing *Acetobacter* dominance in fly-conditioned ones. Finally, interactions among gut bacterial members were studied using *Lactobacillus* and *Acetobacter* isolates, obtained from the host gut. We observed antagonismas well as growth promotion among the isolates, suggesting complex interactions. In conclusion, this study confirmed that genotype and diet contribute to the modulation of the gut microbial community of the fruit fly *D. melanogaster*.

P116

Salt application highlights the relevance of genotype x genotype interaction in the nitrogen fixing symbiosis between *Sinorhizobiummeliloti* and the host plant alfalfa

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Degradation of soil quality due to salinity is one of the most severe and widespread problems affecting agricultural productivity in arid and semi-arid areas. The progressive salinization of soil strongly influencesthe symbiotic interaction between alfalfa and their associated rhizobia affecting the early stages of the symbiotic process. Successful symbiotic N_2 fixation under salt stress can be obtained if both partners resistsuch stress. Generally, alfalfa cultivars are considered more sensitive to salinity than their rhizobial partner, highlighting how the choice of saline-tolerant cultivars is a key factor for high legume yield in saline soils. However, the selection of NaCl-tolerant rhizobia is still fundamental for increasing alfalfa yield in saline conditions. With this aim, extensive isolation and identification of *Sinorhizobium meliloti* strains from alfalfa root nodules in four different sites in Algeria were performed. Also, the efficiency of the symbiotic pairs *S. meliloti* - *Medicago sativa* was evaluated at 0 mM and 100 mM NaCl in controlled conditions. The strains able to efficiently improve plant growth at 100 mM NaCl are different and specific for each cultivar. Though plant cultivar and rhizobial strain significantly impacted plant phenotypes, confirming the importance of strain and plant genotypes over symbiotic phenotypes. PERMANOVA indicates that the genotype x genotype interaction is more relevant in unmasking difference in plant phenotypes when the plants are grown under salty conditions than in unsalted controls. Moreover, obtained results allowed to identify 5 strains as candidate inoculants tested in a field more similar to natural conditions, with induced osmotic stress due to the absence of irrigation.

P117

Enhancing nitrogen fixation for sustainable crop production: harnessing the potential of PGPRs

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Biological nitrogen fixation (BNF) is a vital process through which prokaryotes convert atmospheric nitrogen (N2) into a usable form (NH4+), benefiting plant nutrition and nitrogen compound distribution in ecosystems. To enhance nitrogen fixation in crops and mitigate negative environmental impacts of chemical fertilizers, Plant Growth-Promoting Rhizobacteria (PGPRs) offer a promising solution. PGPR strains were isolated from tomato rhizosphere samples obtained from the Kati region in Mali using a nitrogen-free enrichment technique, followed by streaking on nutrient-rich media for pure culture isolation. Taxonomic classification relied on 16S rRNA gene sequencing, while NifH gene amplification measurements were used to determine nitrogen fixation ability. Further *in vitro* assessments included bacterial biomass production and acetylene reduction assay (ARA) under nitrogen-limited conditions. The best-performing strains will be evaluated in planta through pot trials, measuring plant biomass production. Metagenomic and untargeted metabolomics analyses will provide insights into bacterial diversity and root exudate composition. Whole genome sequencing of the PGPR strains will allow a comprehensive analysis of their genetic potential and functional traits. Currently, 25 rhizobacteria strains have been isolated and are under examination for selection in the pot trials. This ongoing research aims to explore the mechanisms of beneficial interactions between PGPRs and plants, identify and characterize novel PGPR strains with superior nitrogen fixation capabilities, and develop tailored bioinoculants for specific crop requirements. This study explore the mechanisms underlying the beneficial interactions between PGPRs and plants, elucidating how these microorganisms can stimulate nitrogen fixation. Additionally, we will showcase cutting-edge research on the identification, isolation, and characterization of novel PGPR strains with superior nitrogen fixation capabilities. This will facilitate the development of tailored bioinoculants that align with specific crop requirements.

P118

Biostimulation of organohalide respiration through food waste substrates: circular economy in remediation interventions

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Groundwater contaminants such as chloroethenes (tetrachloroethene, PCE, trichloroethene, TCE, dichloroethene, DCE, and vinyl chloride, VC) affect high-quality water availability worldwide. They undergo reductive dechlorination through organohalide respiration (OHR) by anaerobic bacteria such as *Dehalococcoides* and *Dehalogenimonas*. Enzymes involved in dechlorination activity are reductases encodedby *pceA, tceA, bvcA, vcrA and cerA* genes. Microbial dechlorination is a valid ally in remediation of chloroethenes contaminated sites and addition of reducing substrates allows to enhance dechlorination activity thus improving remediation efficiency. Biostimulation with food processing residues may be a bioremediation strategy that allows to fuel anaerobic microbial food web, allows to reduce environmental impact of bioremediation measures, and it is in frame with circular economy concepts.
Since knowledge of the effectiveness of such substrates on OHR microbial communities of anaerobic groundwaters is still scarce, the aim of the present work was to investigate the effect of three substrates from food wastes (engineering molasse, by-product of lycopene extraction and whey) to enhance OHR in a 150- 300 mg L/1 chloroethene-contaminated groundwater. As determined by GC-MS analyses, biostimulation effect was relevant during the first 4 months of incubation resulting in a less accumulation of VC. OHR biomarkers for *tceA, vcrA* and *Dehalococcoides* 16S rRNA were detected in the range of 104-107 gene copies mL/1. The impact of substrates on microbial community is under investigation by 16S rRNA Illuminalibraries.
Food wastes proved to be efficient in biostimulation of OHR activity thus envisaging an increased sustainability of bioremediation intervention in a circular economy frame.

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P119

Distinctive alterations in metabolome and gut microbial community structure of *Eisenia andrei* earthworms induced by co-exposure to environmental microplastics and the pesticide 2,4-D

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Microplastics (MPs) are recognized as emergent pollutants and are considered a relevant environmental issue, particularly when they are associated with other contaminants. In this research, earthworm *Eisenia andrei* was exposed to microplastics (10 µg/kg of soil), the herbicide 2,4-D (7 mg/kg of soil), and the mixof the two for 7 and 14 days. The study quantified the chemical loads in earthworms and examined the bacterial and archaeal diversity in soil and earthworm gut, as well as the gut metabolomic profile.
Furthermore, an integration of multi-omic data was performed to correlate changes in gut microbial diversity with different metabolites. The results demonstrated that earthworms ingest MPs, leading to increased 2,4-Daccumulation. High-throughput sequencing revealed a shift in microbial diversity depending on single or mixed exposure. Metabolomic data demonstrated significant alterations in metabolites related to oxidative stress, inflammatory system, amino acid synthesis, energy metabolism, and DNA metabolism, with more pronounced effects observed in cases of co-exposure. Collectively, this investigation reveals that the combined exposure to MPs and the herbicide 2,4-D poses potential risks to earthworms and, consequently, soil fertility, expanding our understanding of the toxicity of MPs and their impacts on terrestrial environments.

P120

Unravelling the impact of plant protection products (PPPs) on non-target organisms: *in vitro* and *in vivo* antimicrobial activity evaluation

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Several Plant Protection Products (PPPs) are authorized as insect pest sustainable management according to the EU Sustainable Use of Pesticides Directive (2009/128/EC). A promising strategy for their application relies on their antimicrobial activity towards obligate endosymbionts, thus impairing insect fitness. Indeed, some PPPs have already been proven to successfully prevent symbiont acquisition. However, the effect of endosymbiont-targeting compounds on the plant/soil biodiversity and non-target organisms should be evaluated to confirm their sustainable use.
In this context, the aims of this study were to evaluate the effect of fungicides (copper oxychloride-Neoram WG®, dodine-Syllit 544 SC®) and fertilizers (copper and citric acid biocomplex-Dentamet®), already used to control some insect pests, on representative strains of the beneficial microbiota associated with strawberry plants and pollinators such as *Apilactobacillus kunkeei*, *Lactiplantibacillus fragifolii* and *Leuconostoc citreum*, through in vitro tests, as well as on the insect model *Galleria mellonella*.
Antimicrobial activity of endosymbiont-targeting compounds was determined through the Minimal Inhibitory Concentration (MIC) assay. For each substance, different concentrations were assessed in 96-wellmicroplates, with three replicates for each strain, and MIC was compared with field dose.
The survival percentage of 15 *G. mellonella* larvae inoculated with each PPP at field and lower rates wasalso calculated, including a placebo inoculum to control the impact of physical trauma.
This study will improve our understanding on the possible side effects of PPPs, which should be able to control target insect pests (e.g., the olive fruit fly *Bactrocera oleae*), without perturbate niche-adapted microbial communities of plants and pollinators, essential for to maintain healthy ecosystem.

P121

Zooplankton influences extracellular DNA fate and uptake throughnatural transformation in freshwater microcosms

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Aquatic environments represent an important reservoir of antimicrobial resistance (AR) determinants, i.e. antibiotic resistance genes (ARGs) and bacteria (ARB). Horizontal gene transfer can mediate the spread of ARGs in the bacterial populations: considering natural transformation, few data are available for freshwater habitats, mainly because of the difficulty in tracking this phenomenon. Since in aquatic ecosystems the zooplankton plays a crucial role interacting closely with bacteria, aim of this work was to investigate its influence on the fate of extracellular DNA (eDNA) and on eDNA acquisition by bacteria through natural transformation. Experiments were performed in microcosms using *Daphnia obtusa* as the model organism of zooplankton and *Acinetobacter baylyi* BD413 as the model of natural competent bacterium. In presence of alive individuals of *D. obtusa*, plasmidic DNA was degraded, as showed by gel electrophoresis and quantitative PCR analysis. Conversely, plasmid conformation changed (following an animal concentration- dependent trend) in microcosms added with the water in which *Daphnia* and its microbiome released compounds among which proteins (i.e. secretome). Proteins released by *Daphnia* and its microbiome, characterized by functions related to DNA binding and degradation, were identified through a LC-MS/MS, suggesting a contribution of *Daphnia* and its microbiome to eDNA degradation. We measured the ability of *A. baylyi* BD413 to acquire plasmidic DNA incubated in presence and absence of *D. obtusa* and in presence of molecules released by the zooplankton. Transformation frequency was low in presence of the zooplankton due to plasmid degradation, while in presence of *Daphnia* secretome we found an increase of transformation frequencies, probably due to the presence of a plasmid conformation more accessible for the bacterial uptake. In conclusion, we showed that zooplankton and/or its microbiota affect eDNA fate and topology influencing its uptake by bacteria, suggesting that it could contribute to the spread of AR in freshwater.

P122

Understanding the biocontrol mode of action of an epiphytic yeastof apple fruits against *Penicillium expansum* causal agent of postharvest blue mould

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Postharvest biocontrol agents are considered a viable alternative to the use of synthetic chemicals as demonstrated by extensive research conducted worldwide. A yeast isolated from apple fruit proved to be effective in vitro and in vivo against *Penicillium expansum*, one of the most important postharvest pathogensof fruits and vegetables and a food safety issue as producer of the mycotoxin patulin. In the present investigation, the strain T1 of *Meyerozyma caribbica* proved to be able to control both fungal growth and patulin accumulation, and, in addition, to greatly affect disease incidence and severity on apples by a mixed mode of action, including both the competition for nutrients, the induction of resistance and the production of antifungal volatiles. Moreover, to evaluate the volatile organic compounds produced by *M. caribbica* in presence of *P. expansum* a HD-SPME GC-MS analyses was conducted. A total of 44 volatile compounds were detected in the DDS con- frontations; thirteen of them not were accurately identified. These 44 compounds belonged to hydrocarbons (3 compounds), carboxylic acids (7), esters (7), aldehydes (5), alcohols (6), ketones (2), terpenes (4), terpenoids (3), furans (1), and others (6). Yeasts showed relevant differences in their volatile profiles, which presented different compounds associated to their growth.
Although further large-scale trials are needed, the selected strain represents a potential interesting biocontrolagent to be applied after harvest.

P123

The use of microbial based inoculants for enhancing the toleranceof tomato plants to different abiotic stress conditions: preliminaryresults

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The global intensification of agricultural practices and climate change cause an increase in biotic and abiotic stresses leading to a significant decline in crop growth and productivity. The plant-associated microbiota is gaining interest in the agricultural sector since it influences plant growth and mitigates environmental stresses. However, although the results of the applying microbial-based products in controlled conditions areencouraging, field application attempts are more variable and unsatisfactory. This work aims to better understand the plant-soil-microbe interaction in mediating stress response through the rhizosphere microbiome characterization of a tomato plant, treated with a microbial-based product following three abiotic/biotic stress. Additionally, we also evaluated the treatment effectiveness on stress response and analysis of oxylipins, secondary metabolites accumulated in response to stress conditions. While the role of oxylipins in the response to biotic stress is well assessed, their role in plant adaptation to abiotic stress conditions is less studied and the research in this area lacks available data. Here, we will present preliminary data referring to the experiments where the tomato plant was subjected to three drought conditions and salt concentration levels. The data obtained will provide useful insights on the: i) threshold levels of stress conditions and the timing that affects the growth of plants; ii) the oxylipins accumulated in these different conditions over time (1 to 21 days) to understand the dynamic of the plant response and the related-plant associated microbiome under stress conditions.

P124

GOOD-Agroecology for weeds: a Horizon Europe-funded project to promote agroecological transition by diverse strategies, including beneficial plant symbionts

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Weeds negatively affect the sustainability of European (EU) farming systems with weed management relying to a large extent on herbicides. The reduction of herbicide use and risk has become major policy targets of EU Farm to Fork strategy, aiming to promote agroecology and the transition to sustainable and resilient farming systems. GOOD is a 4-year project adopting multidisciplinary approach, aspired to create and evaluate Agroecological Weed Management (AWM) systems, and demonstrate that AWM adoption enhances sustainability and resilience of cropping systems. The main ambition is to foster the agroecologicaltransition for weed management across Europe and beyond. This objective will be achieved through the development, evaluation and demonstration of innovative AWM combinations using cover crops, beneficial microorganisms, such as arbuscular mycorrhizal fungi (AMF), and digital tools for agroecological weed manipulation and management in Living-Labs, co-created with stakeholders in 6 EU pedoclimatic conditions in both annual and perennial crops and in conventional, organic and mixed farming systems.

Native AMF from 7 EU countries will be isolated and reproduced in the Soil Microbiology Labs of the University of Pisa, and utilized for cover crops seed inoculation, to enhance their ability to compete with weeds. A digital AWM Toolbox will be developed to assist farmers' decision making to increase their income and crop productivity. The development and combination of innovative and socioeconomically validated sustainable agroecological practices will generate social, economic and environmental benefits through the reduction or elimination of chemical inputs and optimized use of natural resources linked to the post EU and United Nation 2030 targets. GOOD will create an AWM Network, inspired by the principles of Planetary Health, inviting agroecology practitioners from all continents to an in-depth dialogue and exchange of knowledge and best practices towards agroecology-based diversified agricultural systems to shape the future of humanity and Earth's natural systems.

P125

Two-stage microbial fermentation of food wastes for biopolymerproduction

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Most of food packaging residues are disposed in landfills every year, creating an enormous amount of plasticwaste, thus increasing the interest in sustainable biopolymers such as polyhydroxybutyrate (PHB) that can be obtained from the photo-fermentation of purple non-sulfur bacteria (PNSB) by using food wastes as substrates.
Hence, the aim of the work was to assess the feasibility of a two-stage microbial fermentation using bread and apple wastes for the PHB production. The first stage was represented by lactic acid fermentation of apple peels, (using *Lactiplantibacillus plantarum* and *Leuconostoc mesenteroides* singly and combined) andbread waste (using the starch-degrading strain *Lactobacillus amylovorus* DSM 20532). In the second stage, the fermented broths were used for the photo-fermentation by selected PNSB strains for PHB production. Preliminary tests were performed for determining the best fermentation conditions of apple peels and bread wastes in order to obtain the maximum organic acid yield. The optimized fermented broths were used to set up photo-fermentation assays in anaerobic conditions. The higher concentration of organic acids in apple peels was obtained after 5 days by inoculating the two Lactic Acid Bacteria (LAB) species together, the fermented substrate was characterized by 4.44 g/L of organic acids and allowed the production of 4.21 ± 0.35 (% w PHB/w dry cells) by *Rhodobacter sphaeroides* PISA7 strain, which corresponds to 6.10 mg of PHB produced. Bread wastes, fermented for two days by *L. amylovorus*, were characterized by 4.09 ± 0.89 g/L of organic acids. The bread wastes photo-fermentation reached a higher PHB production, up to 11.01 ± 0.81 (% w PHB/w dry cells) which corresponds to 0.50 g of PHB produced. Although several conditions can be optimized, this study represents a first step for the possible application of a two-stage microbial fermentation process in order to reuse food wastes to produce biopolymers used for food packaging production.

P126

The influence of plant flavonoids on rhizocompetence traits of the polychlorinated biphenyls-degrading bacterium *Paraburkholderiaxenovorans* LB400: a promising resource for rhizoremediation

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Plant secondary metabolites (PSMs) play a key role in the crosstalk between plants and root microorganisms. Previous works unveiled flavonoids' role as biostimulators of the degradative metabolism in polychlorinated biphenyls (PCBs)-degrading bacteria, potentially alleviating the stress caused by these phytotoxic contaminants and strengthening rhizoremediation capabilities.
Plant flavonoids were investigated to assess their influence on rhizocompetence traits of the PCB-degrading bacterium *Paraburkholderia xenovorans* LB400, which are essential features to establish a stable association with the plant and provide beneficial services. Naringin, naringenin and quercetin promoted LB400 growth in terms of bacterial biomass accumulated at stationary phase (+28 %, +17 %, and +40 %, respectively) compared to the control and naringin increased bacterial growth rate by 13 %. Some PSMs also improved functional traits, potentially sustaining the bacterial cell to be recruited by the plant and adhere to the root: naringin enlarged the diameter of swimming motility halo by 7 % compared to control, while quercetin showed a positive effect on bacterial chemotaxis. Moreover, naringin and naringenin had a role in increasingbiofilm formation of the strain (+48 % and +44 % CV/OD600, respectively).
In vitro experiments showed that LB400 colonizes roots and promotes plant growth, even under PCB stress. The colonization pattern of a *mScarlet*-labeled strain on *Arabidopsis thaliana* roots showed that colonizationoccurred mainly on the elongation zone and the apical region and strain re-isolation indicated a 10^8 CFU/mg colonization efficiency at day 14. The bacterium increased root length by 4 % and 8 % in PCB-exposed and control plants, respectively, as well as lateral root density by 72 % in PCB-exposed plants and plant fresh weight after 14 days (+109 % ± 44 % in control plants and +40 % ± 5 % in stressed plants).
These findings contribute to expand the knowledge concerning the crosstalk between plants and beneficial bacteria mediated by PSMs, with the perspective of improving rhizoremediation strategies.

P127

Bioprospecting rhizosphere microbial communities associated withplants living in Mediterranean coastal sand dunes for enhancing plant drought tolerance

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Climate change and global warming have contributed to increase terrestrial drought, causing serious negative impacts on agricultural production and posing severe threats to worldwide food security. Drought stress may be addressed by using beneficial soil microorganisms associated with plant roots, able to improveplant water-use and nutrient uptake efficiency, such as arbuscular mycorrhizal fungi (AMF), that establish mutualistic symbioses with the roots of most food crops and the bacteria strictly associated with their spores and mycelium. The aim of this study was to isolate and characterize AMF associated with the xerophytic plant *Ammophila arenaria*, adapted to the extreme environment of maritime sand dunes, and mycorrhizosphere bacteria, in order to select the best performing strains. The dominant AMF species retrived were *Acaulospora scrobiculata*, *Glomus* sp., *Racocetra fulgida*, *Racocetra persica*, *Racocetra* sp., *Scutellospora* sp. Bacterial strains, selectively isolated for resistance to desiccation, rhizosphere competence and functionally characterized for PGP traits, showed multifunctional properties, suggesting that, acting in synergy with AMF, they may provide additional benefits, improving the performance of drought-stressed plants. In particular, bacteria producing EPS may be crucial for plant performance during drought, as EPS are able to condition the rhizosphere microenvironment and to favour water retention, thus protecting roots against desiccation. Among the bacteria identified, members of Actinobacteria were regularly retrieved from all AMF isolates, confirming their widespread occurrence in the mycorrhizosphere as promoters of AMF activity and functionality. Interestingly, diverse bacterial communities were associated with the different AMF, although originated from the same host plants and environmental conditions, showing that each AMF isolate recruits on its spores a different microbiota. This work makes available AMF and bacterial isolates to be further tested in the formulation of effective microbial consortia, able to positively affect water and nutrient use efficiency, in turn enhancing crop productivity and resilience toward drought stress.

P128

Diversity and composition of soil microbial communities in olive orchards as affected by cover crops, season and natural grass cover

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Soil microorganisms play fundamental roles in the maintenance of biological fertility in agricultural soils, delivering important ecosystem services, such as the completion of biogeochemical cycles and the improvement of soil structure and plant nutrition. The use of cover crops represents a sustainable soil management practice able to maintain crop productivity and to enhance the functional activities of beneficial soil microbiota. This work investigated the impact of cover crops on the biodiversity of soil microbial communities, compared with natural grass cover, in a Mediterranean olive orchard. Soil samples harvested in two successive years were analysed by PCR-Denaturing Gradient Gel Electrophoresis of partial 16S rRNA gene and sequencing the dominant bacterial and fungal taxa. Cluster analysis, non-metric multidimensional scaling (NMDS) and ANOSIM revealed significant differences in the composition of bacterial communities between the two sampling times (R=0.7037 e p=0.0198) and between natural grass cover vs. cover crops (R=0.61111e p=0.0301). The diversity of soil bacterial communities of the two treatments was confirmed also by biodiversity indices: *Shannon* index significantly increased (P=0.0008) in samples from the cover crops trial, compared with the natural grass cover, as well as the indices *Hill1* (P=0.004) and *Hill2* (P=0.0006). Proteobacteria and Acidobacteria were the dominant *phyla* in all samples. Also, for fungal communities, ANOSIM test showed significant differences in the composition of fungal communities between both the two sampling times (R=0.51852 and p=0.013) and the two treatments (R=0.61111 and p=0.01). Results obtained from NMDS showed that the composition of fungal communities diverged mainly on the basis of orchard soil management and secondly on sampling time. Present results show that the use of cover crops in olive orchard management is a better option not only in comparison to tillage, but also to natural grass cover, to maintain and improve soil microbiological diversity and fertility.

P129

Unveiling the interplay between beneficial bacteria inoculants andthe endophytic community of *in vitro* micropropagated plants

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The use of plant probiotics can improve the growth, health and productivity of the holobiont. The beneficial effect of plant growth promoting (PGP) bacteria can be direct or can result from their interactions with the plant-associated microbial community.
This study investigated the response of the plant holobiont to the administration of beneficial bacteria, using micropropagated grapevine plants obtained via somatic embryogenesis. This simplified system (i.e., virus- free specimens with an identical genetic background) allows to assess the inoculants' invasion, their effect on the plant health and to decipher their biotic interactions with the pre-existing endophytic microbiome.
From a collection of endophytes, we selected the most promising PGP strains, *Rhizobium* sp. GR12 and *Kosakonia* sp. VR04, for molecular tagging and the inoculation of micropropagated cuttings. Plantlets were grown in vitro under optimal growth conditions or on a diluted medium to mimic nutritional deficit. Plant biomass was then measured to evaluate the strains PGP activity, and the plant colonization was assessed through qPCR amplification of the marker genes from the DNA extracted from plant tissues. Both the bacterial strains successfully colonized the plant endosphere, and *Rhizobium* sp. GR12 improved the development of the root system of plantlets grown under nutritional deficit, compared to the non-inoculatedones.
The endophytic community of micropropagated grapevine plants was described by high-throughput 16S rRNA gene sequencing and cultivation based analyses. The composition of the endophytic community wasdifferently modulated by *Rhizobium* sp. GR12 and *Kosakonia* sp. VR04 and specific taxa were enriched ordepleted in response to the invasion by these bacteria, reflecting the different plant response in terms of growth promotion.
Our results unveiled the previously hidden diversity of endophytic community in micropropagated grapevine plants and confirmed the importance of the interplays between the plant microbiome members and their dependence upon the plant growth conditions.

P130

Passive biosorption and enzymatic reduction in *Serratia* and *Rhodococcus* strains involved in nickel, copper and chromium removal from wastewaters

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Heavy metals (HM) accumulation in waters represents an environmental and human health threat. HMs are used in industrial processes (industrial welding, dyes and pigments manufacturing, electroplating processes, leather tanning, wood preservation). Microorganisms interact with metals by passive adsorption processes and/or active enzymatic reactions depending both on metal valence and bacterial strain. For this reason, bacteria can be proposed as actors in bioremediation strategies. In the present work microbial mechanisms underpinning nickel (Ni), copper (Cu) and chromium (Cr) resistance were investigated in view of their exploitation in water decontamination processes.
Passive adsorption was the relevant Ni and Cu resistance mechanism for two exopolymeric substances (EPS)-producing *Serratia plymuthica* strains SC3I(2) and As3-5a(5). They were able to remove 89.4 % of Ni(II) (33.5 mg/g d.w.), and 91.5 % of Cu(II) (80.5 mg/g d.w.), respectively. The strains were able to remove metals from real electroplating effluents: SC3I(2) and As3-5a(5) removed 8 and 30 % of Cu(II) from Cu- and 14 and 27 % of Ni(II) from Ni-contaminated wastewaters. Active enzymatic reactions were involved in Cr(VI) resistance in *Rodococcus quingshengii* strain SC26 (MIC of 300 mg/L). In actively growing-cell experiments, 51.14 mg/L Cr(VI) were converted to less toxic Cr(III) during exponential growth phase.
Simultaneously, biomass was able to remove 1.9 mg/g d.w. Cr(VI) by adsorption, being this process less relevant than enzymatic reduction. In order to optimize cell viability and structure to increase interactionwith metals, flow cytometry has been assessed by Fluorescein-labeled lectins specific for strain EPS composition as determined by NMR analysis.
The obtained results evidenced that specific inoculants are required to recover HM from industrial wastewater. Bacterial strains characterization will provide information regarding their possible exploitationin full-scale bioremediation systems.

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P131

Activity and diversity of mycorrhizal symbionts associated withurban trees as affected by pavement treatments

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Arbuscular mycorrhizal fungi (AMF) establish beneficial symbioses with the roots of most land plants, including food crops and shade trees. They promote plant growth and nutrition and improve plant tolerance to pathogens and abiotic stresses, such as drought, salinity and adverse growing conditions. In this work we studied the activity and diversity of AMF communities colonizing the roots of the shade trees *Celtis australis* and *Fraxinus ornus*, frequently grown in urbanized sites as affected by four types of pavements: impermeable monolithic asphalt, permeable modular interlocking pavers, permeable concrete and unpaved soil. To this aim, we assessed the mycorrhizal status and colonization of the root systems of the two tree species, investigated the composition of native root AMF communities by using PCR-denaturating gradient gel electrophoresis (PCR-DGGE) of partial 18S rRNA gene, and identified native AMF by amplicon sequencing. In *C. australis* mycorrhizal colonization showed significant differences among the treatments, with decreases of 58 and 50 % in plants grown in impermeable pavement and permeable pavers, respectively, compared with unpaved control. PCR-DGGE and cluster analyses differentiated AMF symbionts of plants growing in impermeable pavement from all the others, while permeable pavements and unpaved soil showeda similar AMF community. A total of 45 AMF sequence types were detected, with *Sclerocystis* and *Septoglomus* as the most abundant genera, accounting for 84 % of the sequences. The predominance of *Sclerocystis* species in the roots of both trees under impermeable pavements indicated their high and unforeseen tolerance towards harsh environmental conditions. Our results detected the fungal symbionts differentially boosted or depressed in the different types of soil sealing, that will be isolated in order to produce the most resilient specific AMF inocula for trees growing in harsh environments, such as sealed soils in urbanized sites.

P132

Mapping antibiotic resistant bacteria in aquatic environment froma wastewater treatment plant to the Po river to assess their relevance in the One Health approach

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Agri-food systems are crucial hotspots for antibiotic resistance diffusion under the One Health perspective. Antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARGs) can enter agri-food ecosystems through wastewater treatment plant (WWTP) effluents released to surface water bodies and used for irrigation purposes. Though it is known that Horizontal gene transfer (HGT) plays a crucial role in the spreading of ARGs, there are important gaps of knowledge regarding the ARB presence and the frequency of HGT events in the environment. In this context, we are investigating the bacterial resistome in surface waters collected at increasing distances from a WWTP located in the Cremona municipality. Here, we focuson the results related to the cultivable fraction of the bacterial communities distributed along the distance gradient, from the WWTP to the Po River. We established a bacterial collection of two-hundreds strains.

Taxonomic identification showed that our collection encompasses, besides faecal-indicator-bacteria, several genera known to be able to interact with plants (e.g., *Acinetobacter*, *Klebsiella*), previously also reported as potential opportunistic pathogens. The antibiotic resistance profile of the strains was initially characterized by disk diffusion test and Minimal Inhibitory Concentration (MIC) analyses using gentamycin (0.5 % of resistant strains), tetracycline (37 %), ampicillin (42 %), erythromycin (57 %), colistin (10 %) and sulfamethoxazole (100 %). The results showed the presence of 5 multi-drug resistant strains. Based on the antibiotic resistance profiling, different strains were selected for i) genome sequencing and ii) the extraction of plasmids exploitable for natural transformation tests, with the aim to investigate the possible transfer of ARGs to recipient bacteria. Furthermore, given that plants can be considered a “bridge” connecting terrestrial and aquatic ecosystems with the human microbiome, we are currently assessing the capability of the selected strains to colonize the plant root systems, where they could represent a source of ARGs for plant-associated bacterial communities.

P133

Natural transformation of *Acinetobacter baylyi* BD413 is affected by sub-lethal concentrations of antibiotics in freshwater conditions

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Antimicrobial resistance (AMR) diffusion is a global health crisis affecting 700,000 people every year. Globalization has changed the spread of AMR determinants in the environment, making the One-Health approach necessary to manage it. Freshwater bodies, in which treated wastewaters converge, are considered an important route of AMR diffusion, especially if water is reused for irrigation. Despite it is well recognised that the presence of antibiotics in the environment could influence the evolution of resistance and the spread of AMR genetic determinants through Horizontal Genes Transfer (HGT), less is known about how natural transformation is influenced by pharmaceuticals residues in freshwater. The aim of this study was to investigate how different antibiotics at sub-lethal concentrations affect natural transformation frequency in microcosms mimicking environmental conditions (i.e., water composition, temperature) typical of freshwater bodies. Transformation frequency was evaluated in *Acinetobacter baylyi* BD413, used as model strain of natural competent bacteria, measuring its ability to acquire the plasmid pZR80-GFP, in presence of different antibiotics provided in a concentration below the minimum inhibitory concentration (MIC). Natural transformation has been studied in microcosms with *A. baylyi* BD413 and pZR80-GFP incubated in artificial lake water (ALW) added with antibiotics. Transformants were selected by plating on selective medium, and by checking the expression of the GFP gene. Results underlined that natural transformation frequency is influenced by sub-lethal concentrations of antibiotics: compared to unexposed control, frequency can differently shift, being higher, lower or not changing in presence of those antibiotics which resistance is respectively carried, not carried on the plasmid or to which the bacterium is resistant .
This study confirms that the presence of antibiotics in the environment, even in sub-lethal concentrations, influences transformation frequency, underlining how anthropogenic activity could boost the spread of AMRin environments used for recreational and agricultural purposes.

P134

A xenobiotic-triggered “cry-for-help” affects plant-bacteria interaction and shapes root niches to accommodate polychlorinatedbiphenyls-degrading bacteria

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The holobiont dynamics in soils contaminated by recalcitrant xenobiotics are poorly understood. The plant can release a “cry-for-help”, a specific root exudation profile that participate in recruiting and sustaining the microbial services for pollutants removal. To unveil the root chemistry under polychlorinated biphenyls (PCBs) stress, a metabolomics analysis was performed to identify the compounds differentially released by *Arabidopsis thaliana* exposed to PCB-18 compared to plants cultivated under control conditions. Five metabolites were identified among the 62 differentially exudated: coumarin decreased its relative abundance under PCB stress, putatively due to its antimicrobial activity that negatively affects the growth of degrading bacteria. Hypoxanthine increased its abundance with the potential to sustain bacterial growth being used as both carbon and nitrogen sources. Moreover, it enhanced biofilm formation ability, putatively contributing to bacterial rhizocompetence traits.

Besides the “cry-for-help” metabolites, flavonoids are key exudates for the bacterial degradation of PCBs. To explore their involvement in the PCB degrader strain *Pseudomonas* JAB1 rhizocompetence, WT and the tt8 flavonoid iper-producing Arabidopsis lines were used. Confocal microscopy unveiled that JAB1 localized particularly in the root cap, a crucial region for exudation, but an unusual colonization site for soil microbiota. This root niche was therefore investigated by microprofiling pH (as a proxy of root chemistry) and redox potential (as a proxy of the bacterial activity) through the Unisense technology and by mapping the oxygen concentration (as a proxy of bacterial respiration) through PreSens flat sensors in WT and tt8 plants, revealing substantial differences in the plant root tip in presence of the bacterium and under PCB stress.

These findings improve the understanding of the holobiont functionality under xenobiotics stress.

P135

Plastic mulch residuals in soil: impact on bacterial and fungal communities and microbial potential degradation

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Microbial communities actively participate in the degradation of plastic fragments in the soil after mulching. Biodegradable plastic mulches may have different effects on soils compared to conventional ones.
The experimental design aimed to evaluate the potential degradation and the dynamics of the microbial population of biodegradable (M-MB) and non-biodegradable (M-LDPE) plastic mulches mixed with soil for a 6-month incubation under different temperatures (room temperature, 30 °C, and 45 °C). Complete biodegradable Mater-Bi film (MB) was used as a positive reference, and no plastic-added soil was used as control. The experiment was conducted in magenta containers with a mixture of soil and plastic residuals ≥ 2cm^2 (1 %, w w-1).
At the end of the experiment, larger plastic fragments were separated and weighed to determine the fraction of non-degraded plastic. The highest percentage of degradations were recorded for the MB film, and-M-MB,while the LDPE sheet did not show degradation.
Bacterial and fungal communities were analyzed using high-throughput sequencing to evaluate changes occurring throughout the trials. The Alpha diversity of microbial communities was influenced by the plastic type and temperature for bacteria whereas the fungal population was affected by temperature. All variables (plastics, temperature, and time) influenced the Beta diversity of bacterial and fungal communities.
Differential abundance analysis paired with the core microbiome identified target microbial ASVs dominating the distinct soil-plastic ecosystems. The bacterial genera *Streptomyces*, and *Nonomuraea*, andthe fungal species *Acrophialophora levis*, *Mortierellaceae* sp, and the *Agaricacea* family were associatedwith soil mixed with M-MB and MB.
In conclusion, the biodegradable plastic residuals were partially degraded in six months, and this degradation was potentially linked with specific microbial populations. These findings suggest the potential for specific microbial consortia to enhance degradation processes, which could be utilized in bioremediation plans for plastic-contaminated soils to promote agroecosystem sustainability.

P136

Exopolysaccharide production, biofilm formation and adhesion as screening criteria for the selection of bacterial inocula functional tothe promotion of plant drought tolerance

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Plant growth-promoting (PGP) are gaining increasing attention for their possible use in bioinoculant formulations for sustainable agriculture. The production of extracellular polymeric substances (EPS) is a key driver of bacterial establishment in the rhizospere and the expression of PGP traits. However, biofilm formation and adhesion to solid surfaces are also important processes when screening for novel candidates, considering their importance for rhizosphere competence. In this work, EPS production, biofilm formation and adhesion capability were studied in 120 bacteria isolated from the fruiting body of *Tuber borchii* through an optimized step-wise protocol. The strains were qualitatively tested for EPS production on agar medium with a 40:1 C/N ratio, on which EPS producers form smooth, mucous colonies. The best performing EPS isolates were further characterized by EPS quantification using alcohol precipitation method and Congo red binding assay. Biofilm formation and matrix overproduction were assessed by Congo red agar (CRA) method. The ability to adhere to solid surfaces was quantitatively determined in 96-well microtiter plates by crystal violet (CV) binding assay. Most strains showed EPS accumulation on solid medium after 48 hours.

The highest EPS production was 3.27-2.43 g of EPS l-1 of bacterial culture. Overall, several strains showed biofilm formation on CRA after 72h, although some showed early (16 hours) and others showed only late (72 hours) biofilm production. The ability to produce biofilm was always accompanied by good/very good EPS productivity. By contrast, CV assay adhesion capacity did not necessarily correlate with EPS/biofilm production, as strains resulting mild/poor EPS producers and late biofilm formers still showed significant adhesion in multiwell plates. Our results showed that although EPS production and biofilm formation are important biological aspects in relation to each other, adhesion capacity should always be included in the screening process and characterization of novel candidate characterized by drought-tolerance traits.

P137

Beneficial yeasts promote the growth of summer squash (*Cucurbitapepo* L.) at early stage of growth

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The use of microbes able to positively interact with plants is of crucial importance to enforce the assumptionof a climate-smart agriculture with a reduced intake of chemicals and, at the same time, aiming to increase the productivity of crops. This implies an effort to optimize the criteria for the selection of potential plant growth promoters even focusing on yeasts, not yet much investigated for their PGP potential. The present study employed a set of Ascomycetous and Basidiomycetous yeasts to test their PGP properties on summer squash (*Cucurbita pepo* L.) grown in pots, chosen as a fast-growing plant with a vast economical interest.
The inoculation of the seeds with yeasts produced a general positive effect on the early phase of growth of the zucchini plants, primarily affecting the root development. The three species *Schwanniomyces etchellsii*,*Zygotorulaspora florentina* and *Holtermanniella festucosa* were able to induce a strong and significant growth enhancement of weight and length of both aerial and hypogeal parts of the plant. Furthermore, the presence of yeasts induced strain-specific alterations of the soil metabolome that followed the trend of the cellular density and are mainly detected in the rhizosphere, suggesting an active interplay between the rootsand the added yeast cultures. Finally, the data presented lead to the conclusion that the addition of organic matter is a key parameter of the inoculation to fulfil its actions.

P138

Dynamics of native AMF communities in maize roots as affected byplant genotype, starter fertilization and a seed-applied biostimulant

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Sustainable intensification of crop production may be implemented by a promising strategy involving the utilization of beneficial root-associated microorganisms, such as plant growth-promoting (PGP) bacteria and arbuscular mycorrhizal fungi (AMF). The aim of this study was to investigate whether a seed-applied biostimulant, containing a PGP strain of *Bacillus amyloliquefaciens*, and nitrogen (N)-phosphorus (P) starter fertilization, can shape the communities of native root-colonizing AMF in two maize genotypes differing for early vigor. A factorial growth chamber experiment was set up in natural soil, and plants were harvested at two time points: at 13 (emergence) and 49 (5-leaf stage) days after sowing. Mycorrhizal colonization of maize roots by native AMF communities at the 5-leaf stage ranged between 5 % and 54 %, with a significant negative effect of NP fertilization, regardless of host genotype. Accordingly, mineral fertilization was the major driver of native AMF communities colonizing maize roots, as assessed by PCR–Denaturing Gradient Gel Electrophoresis separation of the 18S ribosomal RNA gene and amplicon sequencing. Here, for the first time, we described significant interactions between conventional NP fertilization and a biostimulant containing *B. amyloliquefaciens* IT-45, that affected native AMF, although the combined effects of the two factors were modulated by maize genotype. Amplicon sequencing allowed the identification of the predominant AMF in maize roots, represented by *Glomus*, *Funneliformis* and *Rhizoglomus* species.
Funneliformis mosseae appeared to be resilient across all treatments in both maize hybrids, while populations of the genus *Rhizoglomus* were more affected by the interaction between microbial biostimulant and NP fertilization. The results of this study increase our understanding of how plant genotypes, PGP bacterial strains and NP fertilization can interact, altering the dynamics of native AMF communities in maize. Present findings pave the way for the implementation of beneficial root-associated microorganisms insustainable and resilient agroecosystems.

P139

Winter cover crop in rice cultivation promotes phosphate solubilizing bacteria

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Despite phosphate (P) abundance in paddy soils (0.2–1 g P kg^{-1}), only 2-6 % is bioavailable for rice uptake. In an agroecological perspective, P supply can be achieved by integrating mineral fertilization with conservative agronomic practices like winter cover crop (CC). Previous studies demonstrated that these practices have positive effects on nitrogen rice nutrition, but little is known about P biogeochemical cycle and related microbiome.

In this study, open fields cultivated with continuously flooded rice (*Oryza sativa* L.) were compared as monoculture and after winter CC vetch (*Vicia villosa* L.). Illumina sequencing of bacterial and archaeal 16SrRNA genes and fungal ITS evidenced that rice and vetch hosted distinct bacterial and fungal communities (PERMANOVA - p value < 0.01). The adoption of winter CC increased species richness and P solubilization functional trait of the microbiome of rhizosphere soil and endosphere, as also confirmed by isolation of 200 PGP bacteria. The best-performing *Pseudomonas koreensis* strain 69RS was able to solubilize mineral phosphates (tricalcium, Fe and Al), mineralize the organic one (phytate), fix atmosphericnitrogen and produce plants growth hormones. The strain significantly increased rice root growth and phosphate availability, being able to colonization plant tissues in pot experiments.

It is possible to conclude that rice improves P acquisition when adopting winter CC also thanks to an increased soil microbial diversity and that P bioavailability is linked to the presence of PGP strains like*Pseudomonas koreensis* strain 69RS.

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P140

Chestnut green waste composting for sustainable forest management

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The chestnut is an important species for nut and wood production in Italy, but it produces large quantities of waste residue resulting in forest fires. Making compost from chestnut waste is a possible sustainable management strategy that employs a high-quality renewable organic resource. In this study, in situ composting processes were developed. End-products were characterized by a high-throughput sequencing to investigate its microbiota and different biometric indices were evaluated to assess the effect on plants development. Antimicrobial activity of the compost and of its fractions (e.g., compost tea) was evaluated in vitro through the growth inhibition of plant pathogens. To assess compost quality phytotoxicity assay was also carried out using seeds of *Lepidium sativum*. Microbiota analysis indicated that Actinobacteria and Proteobacteria dominated the compost samples accounting for approximately 60-70 % of the total biodiversity, followed by phyla Planctomycetes, Chloroflexi, Acidobacteria, Firmicutes and Gemmatimonadetes. Exploring the fungal diversity, only three phyla (Ascomycota, Basidiomycota and Mortierellomycota) were detected in all compost samples with an abundance > 1 %. The germination index was 1.2-1.7 indicating no inhibition of germination. In tomato grown on 50 % compost substrate, a general modification of the growing habitus was observed, but more relevant, enhanced resistance to *Phytophthora infestans*. In *Lactuca sativa* was observed an increase in try weigh as well as in resistance to *Phytophthora cryptogea*.
The composting of chestnut waste may represent a sustainable agricultural practice for disposing of lignocellulosic waste by transforming it into green waste compost. Chestnut compost obtained from chestnut residues shows all the characteristics to be classified as green compost and it could be considered a high- quality product.

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P141

Exploring the rhizosphere microbiota to obtain new biostimulantmicrobial consortia for agricultural system

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Due to their role into biogeochemical cycles of relevant soil nutrients, healthy soil microbiomes are crucial for achieving high productivity in combination with crop quality. Plant species could influence soil microbial diversity and vice versa. In this study, a high-throughput sequencing approach was used to investigate the endophytic microbiome associated with tomato plants to identify relevant microbial taxa that could be potentially used to develop an innovative microbial biostimulant. Two different tomato cultivars were cultivated in the southern Italy in open field conditions and subjected to conventional or organic management. Eight replicates for each condition were sampled at different growth stages (post-transplant, flowering, and harvesting). Bioinformatic analysis has identified 11 bacterial genera (*Bacillus*, *Streptomyces*, *Pseudomonas*, *Devosia*, *Agrobacterium*, *Lechevalieria*, *Variovorax*, *Sphingobium*) able to colonize the rhizosphere of tomato plants. It was interesting to note that the relative abundance of different taxa was affected by the plant growth stage. In particular, the genera *Cellvibrio* and *Salinibacterium* were found at post-transplant stage, whereas *Pseudonocardia*, *Stenotrophomonas*, *Lechevalieria*, *Variovorax*, *Flavobacterium* and *Pseudomonas*, known for their plant growth promoting traits, were mainly present at latter stages indicating their possible involvement in plant development. Exploring the fungal diversity, nosignificant differences based on the phenological plant stage were observed.
The innovative approach used for the exploring the endophytic microbiome is useful to identify the microbial taxa particularly adapted to this environment and able to colonize tomato root to isolate microbial species with high potential plant growth promotion activities to develop an innovative microbial biostimulant.

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P142

Delivery of plant-growth promoting bacteria embedded in a bio-based material derived from food waste biomasses

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The amount of food losses along the supply chains represents an evident, ethical, and environmental problem. Within the agri-food sector, the replacement of plastic plantlet containers with bio-based configurations derived from food wastes has the potential to increase the overall production sustainability. Bio-based nursery pots can be directly placed in soil, decreasing transplant stress and labour, and avoiding plastic waste generation. Moreover, these containers can be embedded with biostimulant agents, to increaseplantlet growth decreasing the fertilizer needs. The aim of this work is to study the delivery of plant growth promoting (PGP) bacteria within a biopolymerobtained from food wastes. Plant-associated bacterial strains were selected based on their in vitro PGP properties and were subjected to additional screenings to test their tolerance to different abiotic stresses. Polysaccharidases activity was also tested to verify the possible strain capacity to degrade the biopolymer. The ability of bacteria to promote plant growth was further assessed on *Lactuca sativa* under greenhouse conditions by inoculating the strains in soil before seedlings germination. *Rhizobium* sp. GR12 increased plant height and the nitrogen-flavanol index. All the tested strains including *Bacillus* sp. LR01 and *Kosakonia* sp. VR04 improved nitrate content in lettuce leaves.
The viability of the bacteria within the polymer was assessed by embedding the strains into the liquid material and by re-isolating them after its solidification and drying. *Bacillus* sp. LR01 was observed to grow from 1 cm2 of material deposited on tryptic soy agar (TSA) medium up to one month of biopolymer storage.This strain was also successfully re-isolated by dissolving the polymer in physiological solution and by plating it on TSA medium. The average living cell concentration spanned between $4*10^3$ and $1*10^4$ CFU/g ofmaterial. Further studies are ongoing to improve the viability of the strains and to test their delivery in vivo.

P143

Development of a starter of native lactic acid bacteria for the production of YoALP® Aosta Valley fermented milk

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The research activity is aimed at enhancing the raw materials and microbial biodiversity of the Aosta Valley, through the use of native lactic bacteria belonging to the Institut Agricole Régional's bacteria strains collection for the production of a fermented milk, named YoALP®, with the characteristics of a yoghurt, increasing interest in a dairy product entirely from the Aosta Valley.
49 bacterial strains belonging to the species *Streptococcus thermophilus* and *Lactobacillus delbrueckii* have been isolated over the years from lactic microflora typical of the Aosta Valley agro-pastoral environment such as mountain pastures and small dairies that traditionally process milk without the use of commercial starters. The selection of the strains was based on the study of the technological and genetic characteristics:

- the genotypic and protechnological analysis allowed a preliminary selection of strains for their uniquephylogenetic profile, as well as for their potential use in the preparation of yoghurt (growth at 42 °C, proteolytic activity, acidifying power, production of aromatic and exopolysaccharide compounds);
- the technological evaluation of Aosta Valley cow and sheep milk, was done through industrialization trials on a pilot scale of the blended strains that presented suitable technological characteristics.

As a result, a lyophilized mixture for direct inoculation was formulated. This led to the application characteristics capable of guaranteeing technological performance and ensuring a high vital microbial load during product storage. The mixture contain two strains of *Streptococcus thermophilus* and a strain of *Lactobacillus delbrueckii* not belonging to the subspecies bulgaricus but with high homology with the ssp. delbrueckii and lactis. Overall, the blend showed excellent technological properties even on vegetable drinks and in the presence of honey, confirming its potential also for the production of alternative food.

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P144

Aosta Valley: a case study of microbial biodiversity

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Aosta Valley is inserted in the Alpine mountains which incorporated various regions of the EU, and Switzerland. The Alpine context represents a remarkable mine of biodiversity, both animal and vegetable. In particular, thanks to a unique agricultural management, a precious *reservoir* of microbial biodiversity is present and its maintained - especially in the context of the food supply chains. The former had allowed the research, the selection and the creation of isolated starters on the territory not only for the production of products GI but also for the production of fermented products typical of the Aosta Valley agro-pastoral environment.
In the context of the production of Fontina PDO cheese and Valle d'Aosta Fromadzo PDO cheese, starters were selected, as required by the production regulations. From the lactic microflora isolated from traditionalspontaneous fermentations - without the addition of commercial cultures - strains of lactic bacteria were studied. They were characterized and selected to be safeguarded in the strain library of typical dairy microflora of the Institut Agricole Régional and also to be used as starter cultures in Aosta Valley dairy production.
Furthermore, a mixture of ferments selected in the area was created. This made possible to obtain YoALP®,a fermented milk, made from Aosta Valley cow and sheep milks which fully exploits the raw materials of the Alpine territory.
Finally, according to parameters of technological interest, for a guided fermentation of the musts, the practise of inoculating selected strains of *Saccharomyces cerevisiae* is widespread. The need emerged that was to isolate, characterize and preserve yeast strains from different viticultural areas of the Aosta Valley, including 9 strains of *S. cerevisiae* tested and analysed through experimental microvinification tests in the cellar. Thanks to this technique were able to develop, the flavours and the aromas typical of the Aosta Valleywine-growing reality.

P145

Epilithic biofilms of acid mine drainage-affected mountain stream: ecological role of arsenic and heavy metal sinks in natural environments

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Mountain areas can be affected by arsenic and heavy metal contamination due to acid mine drainage from abandoned mines.
This work aimed at analyzing the microbial composition and metabolic strategies of epilithic biofilms in a natural freshwater creek to unveil their ecological role as natural sinks of metals, to evaluate possible exploitation in bioremediation.
Epilithic biofilms within an abandoned gold mine and 1.7 km-downstream the mine were characterized by Illumina 16S rRNA gene amplicon and shotgun sequencing. Putative functions as per genomic data were paralleled by cultivation and characterization of heterotrophic and autotrophic bacteria involved in arsenic biogeochemical cycle.
Mine and downstream biofilms were affected by arsenic contamination (93.43 and 8.66 g/kg d.w.). Other heavy metals accumulated in downstream biofilm (3.59-10.00 g/kg d.w.) and to a lesser extent in creek freshwater (0.104 mg/L). Mine biofilm characterized by pH 2 was dominated by acidophilic iron- and sulfur- oxidizing microorganisms belonging to *Acidithrix, Acidiphilium* and uncharacterized *Planctomycetota*. Sub- neutral pH 6.7 in creek freshwater promoted the establishment of a significantly more diverse community driven by *Cyanobacteria* in downstream biofilm. Metagenomics and strain culturomics revealed that arsenic resistance was mediated by the ARS operon for arsenate reductase and arsenite efflux pump. To a lesser extent, arsenite oxidation was occurring *via* arsenite oxidase. The isolation of autotrophic arsenite-oxidizing bacteria/cyanobacteria confirmed this feature. Extruded arsenic inorganic forms were embedded in the extracellular polymeric matrix.
These outcomes revealed that acid mine drainage-affected epilithic biofilms are natural sinks for arsenic and heavy metals, characterized by the assemblage of bacterial species with relevant role in decontamination of natural environments and with a great potential for arsenic and heavy metal bioremediation in freshwaters.

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Poster Session 4

Exploiting microbiomes for a sustainable future

P146

Lactiplantibacillus plantarum strains as growth promoters and elicitors of responses to biotic and abiotic stresses in horticulturalspecies

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The interest in the employment of biocontrol agents as an alternative to chemical agents to manage phytopathogens and to promote plant growth has increased in recent years.
Lactic Acid Bacteria (LAB) have been suggested as potentially useful bacteria against phytopathogens with plant-promoting capabilities and their safety level makes them attractive for use in sustaining crop protectionand productivity.
In the present study, five strains of *Lactiplantibacillus plantarum* from different sources (cheese, olive, cereals) were evaluated by phenotypic tests to select PGP strains with plant growth promoting activities under stress conditions and protection against phytopathogenic fungi. All the strains complied with EFSA'srequirements with regard to the antibiotic resistance profile.
Seed treatment with LAB strains did not affect the quality of seed germination and seedling vigor evaluatedboth by in vitro test and under green house in tomato plant, lettuce, and radishes.
The five strains of *L. plantarum* completely inhibited mycelial growth of phytopathogenic fungus *Botrytis cinerea* Pers. strain 2N15 under in vitro tests, and two out of five were effective against *Fusarium verticillioides* (Sacc.) Nirenberg strain FV2221, whereas none of the strains inhibited the growth of *Fusarium graminearum* (Schw.) strain FG515.
Furthermore, the secretomes of the five bacterial strains were characterized using a shotgun proteomics approach to get insight into strain diversity and the 2 most effective strains were subjected to Whole Genome sequencing.
In vivo test will be performed to assess the efficacy of LAB treatment to control fungal plant diseases andthe promotion of horticultural plants growth under abiotic stress conditions.

P147

Investigating the potential of yacon *(Smallanthus sonchifolius)* juice in the development of organic apple-based snacks

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Yacon syrup, comprised of Fructooligosaccharides (FOS), inulin, and a small amount of free sugars, presents itself as a potential nutraceutical product. Its high potential for use in food technology and chronic diseases prevention, especially as a novel source of prebiotics, has been demonstrated in vitro and in vivo through selective fermentation by bifidobacteria and lactobacilli. The aim of this study was to investigate thefeasibility of using yacon juice for impregnating apples to formulate dried organic apple fruit snacks with heath-promoting properties. The technological process was evaluated by examining the microbiological, prebiotic and technological characteristics of the snacks, including during a storage period of 50 days at room temperature. Vacuum impregnation and thermal dehydration processes resulted in yacon juice- impregnated dried apple slices with good technological and microbiological stability. The higher amounts of inulin increased the prebiotic property of the apple slices and promoted the growth andviability of cells in the simulated intestinal fluid of *Bifidobacterium animalis* subsp. *lactis* BB-12, *Bifidobacterium breve* DSM 20091, *Bifidobacterium longum* subsp. *infantis* DSM 20088, *Lactobacillus rhamnosus* GG and *Lactobacillus rhamnosus* C112, even after 50 days of storage. As the physico-chemical and technological parameters remained constant and similar to controls, vacuum impregnation has the potential to be used to produce enriched organic prebiotic apple snacks with increased benefits for consumers.

P148

Arcobacteraceae: genome adaptation and genome size reduction inspecies isolated from animals and human demonstrated by comparative genome analysis

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The Arcobacteraceae bacterial family includes Gram-negative species previously belonging to Campylobacteraceae. Part of these species are considered foodborne pathogens, and are present on different foods and animals. *Arcobacter butzleri* and *Arcobacter cryaerophilus* are the two most isolated species form human clinical samples, and have been isolated from food of animal origin, including chicken and pork. Thedifferent abilities of the Arcobacteraceae to survive in various hosts and environmental conditions suggest an evolutionary pressure linked to genome adaptation. The different physiological and genomic characteristics of Arcobacteraceae species led to the proposal to create new genera, which is however criticized for the lack of discriminatory features and biological/clinical relevance.
The aim of this study was Arcobacteraceae pangenome evaluation in order to characterize possible relationships between 20 validly described species. The analyses have been conducted on type strains genomes obtained by Illumina sequencing. These species were included in different groups considering the isolation sources and information present in literature. The use of different bioinformatics tools enabled the obtainment of information about pangenome partitions (Roary, Panaroo, PPanGGOLiN) and gene classes (EggNOG). The results do not support the proposed division into different genera of the Arcobacteraceae family showing pangenome partitions (core genes) like *Campylobacter* genus. A smaller genome size of theanimal related species was observed suggesting an evolutionary adaptation of these species to hosts.
Moreover, the gene class compositions in animal and human-associated species showed a higher percentage of virulence-related gene classes such as cell motility genes. Some orthologues like MotA/TolQ/ExbB proton channel family (TonB-related; virulence functions), were positively correlated to the animals related species suggesting a specific function of different orthologues. The genome analysis identified the presence of specific genes linked to different species groups. A division into pathogenic and non-pathogenic species issuggested, supporting future research on food safety and public health.

P149

Shelf-life extension of leavened bakery products by using bio-protective cultures and type-III sourdough

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Physicochemical and microbiological alteration of processed foods limits their shelf-life. Spoiled goods are on the watch list of industries interested to identify innovative strategies allowing for shelf-life extension andavoiding changes of safety and organoleptic features, being involved in food waste and economic loss matters. To extend the shelf-life of two different types of bakery products, we evaluated the effectiveness of a type-III sourdough (tIII-SD) combined with a mixture of probiotics (*Lactobacillus acidophilus*, *L. casei*, *Bifidobacterium* sp., *Bacillus coagulans*) that were used as bioprotective-cultures (BCs). This innovative (I) dough was used to produce fresh base-pizza (BP) and focaccia (FO) that were inspected by a multi-omics approach aimful at monitoring features at different time-points of the shelf-life. Differences in physicochemical, protein, microbiological and volatile profiles were also investigated after 10-days of extended shelf-life. The addition of BCs and tIII-SD left unchanged the proximate composition. This, together with the absence of detected microbial contaminations, indicated the suitability of both I-BP and I- FO despite the shelf-life extension. Both I-samples accounted for a more stable and heterogeneous microbiota during the storage phase and showed lower scores of *Alternaria infectoria* and *A. alternata*. In I- samples, volatilomics showed an increased relative concentration of volatile carboxylic acids. Therefore, without resorting to chemical preservatives, the addition of BCs and tIII-SD led to specific microbiological and metabolite improvements in both BP and FO products, whose shelf-life was extended by 10-days under MAP. Shelf life extension can play a key role for reducing food waste and is agreed as an effective way to increase the sustainability of food systems contributing to achieve both the Sustainable Development Goals and the goals of the Paris Agreement on climate change.

P150

Microbial consortia as sustainable biofertilizers: global analysis of their impact

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High temperatures, increased frequencies of heat waves, torrential rain or long periods of drought are all negative events linked to climate change. Actual agricultural practices, based on intensive cultures, do not meliorate resilience of plants to these emergencies. Moreover, the extensive use of nitrogen and phosphorus- based fertilisers determined several negative effects on the agricultural ecosystems, human health, with a dispersion of inorganic and organic contaminants (water eutrophication). In search for more sustainable alternatives, researchers are focusing on biofertilizers, both bacteria and/or fungi (also called Plant Growth Promoting Microbes PGPM) which positively stimulate plant growth and health by promoting nutrient uptake capacity, and the "plant immune system". The use of PGPM can be improved when they were simultaneously supplemented with soil amendments as biochar; the latter is obtained by the heat treatment of agricultural and/or food processing residues, supporting the "end of waste" goal. This combination can improve soil quality and fertility, favour water and nutrient retention and supports indigenous soil microbial population. Therefore, PGPM plus biochar can be considered a new environmental biotechnology to be applied in agricultural ecosystems.
Studies on soil and plant effects of these supplements were performed to the level of bacterial and fungal rhizospheric populations, accompanied with physiological and agronomical data, along with the study of the effects on grain storage proteins. The results obtained demonstrated that bacterial phyla and classes were stable over time and resilient to the treatments given. Fungal population demonstrated to be less stable and varied according to year and cultivar. Exploitation of a new biotechnology should positively have an impacton the future. In this case, the proposed approach is based on the combination of natural biofertilizers (PGPM) and an amendment (biochar) obtained by circular economy to reduce chemical inputs and increase sustainability.

P151

Lactuca sativa genotype influences the plant phenotypic and metabolic response to a soil microbial *inoculum*

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In the last few years microbial-based approaches have been proposed as a possible solution to decrease the use of chemical and non-renewable fertilizers. Among these, the most promising candidates are arbuscular mycorrhizal fungi, with their ability to dramatically extend the root surface involved in the phosphate absorption, and phosphate solubilizing bacteria. The aim of this project is to study the effect of a microbial inoculum, made by two arbuscular mycorrhizal fungi and two phosphate-solubilizing bacteria, on a panel of 128 fully sequenced varieties of *Lactuca sativa* in a controlled condition of P starvation, keeping in mind thatplant genetic diversity plays an essential role in the responsiveness to microbial *inocula*. Using a combination of physiological, metabolic and biomass parameters we were able to reconstruct the plant response to the inoculum. Considering all the lettuce genotypes analysed, about 10 % of these showed a statistically significant effect on plant growth and/or plant phosphate concentration, while the whole panel show a range of different responses highlighting the central role of genotype x environment interaction. *L. sativa* genotypes showing contrasting phenotypes will be further analysed for their photosynthetic pigments, free amino acids, and sugars content and will be linked to their root microbial community. All the phenotypic traits collected during the experiment were used to perform genome wide association studies (GWAS).
Preliminary results demonstrated that SNPs located in chromosome four of *L. sativa* genotype are associatedwith differences in soluble phosphate accumulation upon soil inoculation. In addition to that, two genetic loci in chromosome two and five, respectively, are associated with the diverse effect of the *inoculum* on shoot biomass. In conclusion, with this study we aim to add a new piece of knowledge on the genetic and physiological mechanisms underlying the establishment and the effects of beneficial interaction between plants and soil microorganisms.

P152

Influence of *Aloe arborescens* supplementation in drying-off dairycows on milk microbiome

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Intra-mammary infusion with long-acting antibiotics at dry off is a long-standing practice in the dairy industry to (i) cure existing infections at dry off, (ii) decrease new infections, (iii) prevent clinical mastitis at the start of next lactation and (iv) decrease the somatic cell count during the early lactation. Over the years, this situation has given the possibility to increase the serious problem of antibiotic resistance. Therefore, it isvery important to control the antibiotic consumption and release of antibiotic residues. During the last few years, to improve the animal health, the general interest has been directed in using plant-based additives with proven nutraceutical properties especially during the drying period. *Aloe arborescens* contains polysaccharides and exhibits anti-inflammatory, immunostimulant, antibacterial, and antioxidant properties. The aim of this study was to investigate the effect of this nutraceutical approach on the bovine milk microbiome. The experiment involved 30 multiparous dry cows divided in three different groups: (1) controlgroup - dry cows following the typical antibiotic treatment and the application of teat sealant; (2) sealant group - dry cows without antibiotic's treatment and with only teat sealant; (3) *Aloe arborescens* supplementation group – dry cow with teat sealant and oral administration of 200 mL of homogenate Aloe arborescens from 7 days before up to 7 days after drying. Milk samples were collected 14 days before dry- off, at drying-off and 35 days after calving. The V3-V4 hypervariable regions of the bacterial 16S gene was sequenced in two MiSeq (Illumina) runs with 2×250-base paired-end reads. The use of *Aloe arborescens* influences the milk microbiome composition, increasing significantly (p-value < 0.01) microbial biodiversityafter calving.

P153

Occurrence and antibiotic resistance of *Arcobacter* sp. isolated from poultry slaughterhouses in northern Italy

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Arcobacter sp. is a bacterial genus belonging to the Arcobacteraceae family, identified as a zoonotic pathogen worldwide. The pathogenicity of this microorganism is underestimated due to the lack of knowledge and misdiagnosis of infection, often attributed to *Campylobacter*. The present project aims to detect the presence of *Arcobacter* sp. in the poultry slaughterhouse and to evaluate its antibiotic resistance (AR). Neck skin (BNS) and caecum (BC) from 49 broiler flocks were provided by breeders located in North Italy during slaughtering processes and superficial samples from the slaughterhouse environment (SE) were collected after cleaning procedures. Culture-dependent and independent methods were carried out to identify and characterize *Arcobacter* sp.. Three hundred and thirty colonies were isolated and 320 *Arcobacter butzleri*, 4 *Arcobacter cryaerophilus*, 3 *Arcobacter cibarius*, 2 *Arcobacter thereius* and 1 *Arcobacter skirrowii* isolates were identified by MALDI-TOF MS (confirmed by species-specific PCR). Biotyping among isolates was performed considering the presence-absence of three virulence-related genes (*irgA*, *hecA* and *hecB*). Based on the molecular profile, the AR of 126 isolates was examined, showing that 67 % of the isolates were resistant to at least one antibiotic. *A. butzleri* isolates from slaughterhouse surfaces were more resistant to antibiotics than those from broilers. Bacterial communities of the broiler neck skin, caecum and environmental swabs were evaluated by metataxonomic analysis based on the 16S rRNA gene Amplicon Sequence Variants. *A. butzleri* was found in environmental swabs (9 %) and neck skin samples (4 %) resulting the only Campylobacterota member uniquely associated to BNS and SE. *Arcobacter* sp. have been isolated from broilers in all production runs and the microbiota analysis revealed possible cross- contamination between carcasses and slaughterhouse surfaces. The AR detected is of great relevance considering the possible transmission of resistance factors and underlying the importance of slaughtering processes optimization to reduce its presence.

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The BIO-Save project: university-business alliance in modern biotechnology approaches for climate change mitigations solutions

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BIO-Save is funded within the Erasmus+ programme for education and training, under the Knowledge Alliance framework. Its main mission is to join the available knowledge in climate protection technologiesand translate them into transnational educational programmes.
The partnership is composed of 4 academic partners and 6 SMEs from 5 European countries. The project activities have developed an on-line blended learning programme (available at the website *www.biosave.eu*) for dedicated courses in biotechnology or as support for already existing courses. Specific outcomes of the training programme have been designed following European standards and a multilingual cloud-based platform with dedicated operational functionality is under preparation. The training programme is addressed to bachelor, master and PhD students, post-docs as well as academic professionals.
The training contents have already been defined with the development of different learning outcomes (LOs): LO1-Reduction of greenhouses gases emission; LO2-Use of energy efficient farming; LO3-Carbon sequestration; LO4-Reduced use of synthetic fertilizers; LO5-Adaptation to abiotic stresses; LO6- Restoration of degraded ecosystems; LO7-Resilient crops; LO8-Agroecosystem responses to climate change; LO9-Crop diversification for climate change resilience; LO10-New technologies and practices; LO11-Conservation of plant genetic resources; LO12-ISO standards. The UNIBO unit is specifically involved in LO8, focused on how global climate change will affect agroecosystems. The effects on crops depend on the combined action of several components, such as CO_2 and ozone levels, temperature, change in precipitation pattern and salinity of soils. Agricultural productivity result from direct effects at the plant level, or indirect effects at the system level, i.e. shifts in nutrient cycling, crop–weed interactions, insect pest occurrence, plant diseases. The use of beneficial microorganisms is discussed to counteract these effects in soil.
In conclusion, the BIO-Save blended learning model develops a competence-based learning strategy offeringskills to students, professionals and institutions by a combination of e-learning technology, self-assessment tests and offline materials.

P155

Combined application of a PGPR inoculant with vegetal meal shapes the rhizosphere microbiota of *C. melo* affecting cantaloupeand soil quality

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The application of biostimulative PGPR can be considered a powerful tool to improve sustainability and furthermore, in order to assure high performance in crops with high nutrient demand, might be enhanced with combined application of vegetal meals as organic fertilizers. Currently the agro-technical approach of PGPR inoculation with concomitant application of the OFDP (organic fertilizer derived from plant material) represents a valid alternative for horticultural crop management since it is able to optimize the phase of small plants transplant, when the inoculated seedlings are inserted onto the transplant holes treated with the OFDP. The aims of this study were to characterize cantaloupe quality and the rhizosphere microbiota of *Cucumis melo* var *reticulata* managed with low input fertilization strategies achieved mainly by inoculation of a commercial product (Spring-Up) and as well as with the application of vegetal meal (VEGAND®).
A field experiment concerning the cantaloupe cropping with microbial rhizosphere assessing and chemical characterization of soils, plants and cantaloupe quality was carried out in Central Italy. Metagenomic monitoring of rhizospheric soils, sampled during the harvest phase, was performed by Next Generation Sequencing (NGS) and by chemical characterization of soil, plant and cantaloupe.
Data obtained show that the *C. melo* rhizosphere microbiome was dominated by bacteria from the phyla Actinobacteria, Proteobacteria, Firmicutes, Acidobacteria and Verrucomicrobia. NGS exploration highlights the evolution of rhizospheric microbiome of *C. melo* treated with vegetal meal and shows that α-diversity (Faith index) was reduced in the trophic niche managed by both biostimulative and organic treatments. Specifically the Firmicutes improved with combined application of products that are able to shape also the Actinobacteria and Proteobacteria communities. Brix grade of cantaloupe was optimised in this condition.
Instead in the soil treated with double application was observed the accidental occurrence of potential animalpathogen as *Mycobacterium arupense* and *Clostridium subterminale*.

P156

Reintroducing lost yeast genetic biodiversity using a classical genetic approach

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Due to their extended domestication over the years, several industrial *Saccharomyces cerevisiae* strains are used in various processes, typically for historical reasons instead of scientific and technological needs. As such, there is still substantial room for the improvement of industrial yeast strains relying on yeast biodiversity. This study endeavors to regenerate biodiversity in already available yeast strains through the innovative application of classic genetic approaches. Extensive sporulation was indeed applied on three different yeast strains, specifically selected for their diverse origins and backgrounds, to elucidate how newvariability was produced. A novel and easy method to obtain mono-spore colonies was established, withoutselection, to reveal the degree of variability generated after sporulation. The isolated progenies were then tested for their growth pattern in defined broths with high stressor levels. A considerable and strain-specific increase in phenotypic and metabolomic variability was evaluated, and a few mono-spore colonies were found to be of great interest for future exploitation in specific industrial processes (i.e. wine making and bioethanol production).

P157

Search for bacteriocin producing genes in the poultry gut associated microbiome to support antibiotic free production in poultry farm

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The increasing demand for antibiotic free poultry production of meat together with the spreading of antibiotic resistance is spurring the research for new and alternative solutions to the use of antibiotics at farm level. Among the different compounds, bacteriocins seem to be a promising option, because of their broad spectrum and mechanisms of action. They are produced by bacteria, archaea, and yeasts and act against pathogens thanks to their action on several pathways, such as DNA synthesis inhibition, sugar uptake inhibition, or membrane disruption through pores formation.

In this work, three poultry farms have been considered to search for bacteriocin-producing genes. The farms were scored based on their performance. Respectively, two farms were assigned as low-performance scores (A, B) and one as high-performance score (C). Overall, 199 samples were collected, and DNA extraction and whole shotgun sequencing were performed. Samples were processed for MAGs construction and AntiSMASH was used to identify the presence of biosynthetic gene clusters (BGCs). Fifteen BGCs were identified: arylpoliene, terpene, ectoine, lantipeptide, siderophore, nrps, type 1 pks, type 2 pks, type 3 pks, other, bacteriocin, bacteriocin lantipeptide, microcin, sactipeptide, and thiopeptides. The last five BGCs listed codify for bacteriocins and constitute the bacteriocinome of our sample pod. The results show a higher presence of bacteriocin and thiopeptides in farms A and C and of bacteriocins and sactipeptide in farm C. A general reduction of the total bacteriocinome is observed at the low-performance sites. At the high- performance one, it is observed an increase of bacteriocinome in the first fifteen days when there is the feeding change and a subsequent slight decrease. Bacteriocinome-species blasting on NCBI unearthed 55 bacterial species, of which 18 were identified as new bacteriocinome-associated species. These species couldhave key uses in the production of new probiotics.

P158

Exploiting the use of a macerating consortium to improve the bio-extraction of hemp fibers

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Hemp fiber is known as a versatile and sustainable natural fiber due to its high production potential. It finds applications in various industries such as textiles, yarns, paper, construction materials, auto parts, and composites. Proper retting process, in which efficient separation of cellulose fiber from the rest of the stem is promoted by indigenous microorganisms able to degrade pectin, is essential for hemp fiber production and quality. This research aimed to enhance the extraction of hemp fiber using a selected consortium of bacteria with pectin-degrading abilities.

Four bacterial strains selected based on their high pectinolytic activity and low or absent endoglucanase activity, constituted the macerating consortium. Its effect was evaluated in a lab-scale water hemp retting system through the study of the dynamic of the pectinolytic group, microbiota, and enzymatic activities. Moreover, several features such as decortication, odor, color, and fiber yield were assessed to determine fiber quality during the process.

Results revealed the optimal retting time, corresponding to an increased pectinolytic activity and a reduced endoglucanase activity. Furthermore, the microbiota analysis indicated that only two bacterial strains of the macerating consortium, *Bacillus subtilis* AT2SB-87-P and *Stenotrophomonas pictorum* ET2SB-711-P, were likely responsible for driving the hemp water retting process, allowing reduction of the time and the improvement of the fiber quality.

In conclusion, the overall results suggested the effectiveness of employing the selected pectinolytic bacterial consortium to enhance the bioextraction of hemp fiber during the water retting process. This advancement holds significant potential to produce environmentally friendly natural fibers.

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Evaluation of antimicrobial activity of plant polyphenolic compounds against bacterial pathogens

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The spread of antimicrobial resistance in pathogen bacteria has led researchers to look for alternatives to antibiotics. A promising resource are considered the plant polyphenolic compounds. Natural products produced by living organisms, included plants, as defense against pathogens and stress factors have always been a source of inspiration for new drugs. Particularly, stilbenoids represent a class of polyphenols largely studied for their antimicrobial effect. Resveratrol is a natural polyphenolic compound belonging to the stilbene family. The aim of this study was to assess the antimicrobial activity of resveratrol-derived monomers and dimers screened as single molecules against a panel of foodborne pathogens including Gram- negative such as *Salmonella enterica*, *Escherichia coli* and *Pseudomonas aeruginosa* and Gram-positive bacteria such as *Bacillus cereus* and *Enterococcus gallinarum*.

The antibacterial activity was carried out through the standard microdilution method for drug susceptibility testing using 96-well plates in a final volume of 200 µL in the presence of 10 increasing concentrations of each antimicrobial, ranging from 0.25 up to 128 µg/mL. Moreover, the biofilm formation of three strains of*Pseudomonas aeruginosa* in presence of these compounds was evaluated by, using the Chrystal violet staining.

The results showed that, in general, resveratrol and its derivatives were more active against Gram-positive rather than Gram-negative bacteria. The highest antimicrobial activity against Gram-positive bacteria was observed for the viniferifuran, a dimer, with MIC values ranging from 4 and to 32 µg/mL for *B. cereus* and *E.gallinarum*, respectively.

Regarding the biofilm prevention, the molecules tested proved to limit its formation in two out of 3 strains of *P. aeruginosa*.

Further investigations are addressed to the mechanisms of action of these molecules and their differencesbetween Gram-negative and Gram-positive bacteria.

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Novel probiotic preparation with in vivo gluten degrading activityand modulatory effects on the gut microbiota

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Gluten has unique and unusual characteristics, making it only partially digestible. It also has a negative impact on a large proportion of the population suffering from coeliac disease or related disorders. Finding solutions to break down gluten during digestion is an ambitious goal of high nutritional and social impact. Here, a randomized double-blind placebo-controlled in vivo challenge investigated the gluten degrading activity of a novel probiotic preparation comprising lactobacilli and their cytoplasmic extracts, *Bacillus* sp. and a bacterial protease. Probiotic/placebo administration to 70 healthy volunteers lasted 32 days, followed by 10 days wash-out. After a preliminary gluten free diet (GFD) to eliminate gluten from faecal material, increasing amounts of gluten (50 mg to 10 g) were administered, each one for 4 consecutive days. Compared to placebo, the faecal material of volunteers fed with probiotics showed much lower amounts of residual gluten. This became more evident with increased gluten intakes. Probiotics also regulated the intestinal microbial communities, improving the abundance of genera, which are pivotal to maintain homeostasis. q-PCR confirmed that all probiotics persisted during intervention, some also during wash-out. Probiotics promoted a faecal metabolome with potential immunomodulating activity, mainly related to derivatives of branched chain amino acids and short chain fatty acids. Our work presents a new probiotic preparation suitable for people suffering from gluten related disorders during GFD and for healthy individuals because it enhances gluten digestion and promotes gut microbiota functionality.

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Bioelectrochemical carbon and nitrogen fixation driven by cathodicbiofilms

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Nitrogen is plentifully available in Earth's atmosphere as N_2 gas but inaccessible for most organisms that rely on diazotrophic bacteria for ammonia acquisition. Bio-electrochemical systems (BES) fuel electroactive microorganisms that fix inorganic elements (e.g., C, N) into cells, and might represent a challenging opportunity for soil fertility improvement.
With the aim to demonstrate that diazotrophic consortia can be enriched with reducing power obtained by anelectrochemical apparatus, an anaerobic microbial consortium was inoculated in BES where stable isotopes ^{13}C and ^{15}N-nitrogen gas were provided. BES were operated for 208 days, at constant cathodic potentials of -0.2 and -0.9 V vs SHE along with controls (non-inoculated and open circuit systems).
Significantly higher charge consumption, cathodic chamber biomass and ^{13}C and ^{15}N accumulation were measured in inoculated -0.9 V polarized BES with respect to controls. RNA could only be isolated from the polarized cathodic biofilm, evidencing that biomass production (i.e., carbon and nitrogen fixation) was fuelled by cathodic electrons. Illumina 16S rRNA sequencing of cDNA showed a highly selected community of electroactive Bacteria and Archaea such as *Paracoccus*, Xanthomonadaceae, *Methanobrevibacter* and *Methanoculleus*.
These outcomes offer new insights on the ecological and physiological mechanisms involved in the establishment of electrophilic microbial consortia in oligotrophic environments, and pave the way for novelbiotechnological applications to improve soil fertility.

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Poster Session 5

Sustainable future has come

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Functional hydrolysates obtained through fermentation of flathead grey mullet by-products using *Yarrowia lipolytica* and *Bacillus* sp.

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Recently fish processing industry has experienced significant expansion and this had an impact on the production of fish waste and by-products. Valorization of these marine materials through biotechnological processes can represent a strategy for a sustainable bioeconomy. The aim of this study, performed within the European Project "NewTechAqua", was to use tailored microbial fermentation as a safe and sustainable technology to obtain a wide variety of added-value compounds. Based on a preliminary screening that allowed to define the best proteolytic and lipolytic microorganisms, two strains of *Bacillus* sp. (B5M and B5C) and two strains of *Yarrowia lipolytica* (YL2 and YL4) were incubated up to 9 days with flathead grey mullet (*Mugil cephalus*) by-products. The growth of microbial strains was followed over time by plate counting *Y. lipolytica* in YPD agar and *Bacillus* sp. in BHI agar. Liquid samples were analyzed after 4 and 9 days of incubation to evaluate the peptide content (OPA assay) and antioxidant activity (DPPH and ABTS assay). The production of volatile compounds was evaluated by SPME/GC-MS technique. Samples collectedat 9 days were also lyophilized and characterized in the same way as liquid samples. All the microorganisms were able to develop in the substrate. The peptide content in liquid samples reached the highest concentration (102 mg/mL) after 9 days of incubation, especially with Bacilli. Strains B5M and YL2 showed also the highest antioxidant activity (around 53-60 %). Yeasts produced mainly alcohols and aldehydes, while samples containing Bacilli were characterized by ketones and alcohols. A longer incubation determined a lower abundance of volatile compounds. Regarding the freeze-dried hydrolysates, those obtained with yeasts showed a better antioxidant activity and a higher acids content. Overall, microbial fermentation of fish by-products represents a promising tool to produce functional and flavoring compounds that can be used as ingredients in the food sector.

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Identification of edible plant-derived antimicrobial peptides through a genome mining approach

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The misuse of antimicrobial compounds has led to challenges like the development of antimicrobial resistance in foodborne bacteria, raising concerns to public health and the food industry. Therefore, finding alternatives to antibiotics, such as antimicrobial peptides (AMPs), has become paramount. Plants are among the first AMPs producers discovered, and they can represent a good reservoir of these compounds.
Furthermore, the increasing availability of genomic data has facilitated the development of genome-based tools, like genome mining, that allow the investigation of plant genomes to seek for AMPs sequences. In light of this, our study aimed to mine the genomes from 10 different edible plant species using two different mining approaches based on small open reading frames with sORFfinder and the prediction of protein sequences using Hidden Markow Models. The outcome of the two pipelines resulted in more than 2000 potential peptide sequences. For the selection of suitable peptide sequences with potential antimicrobial activity, different physicochemical parameters were considered: isoelectric point, net charge, pH, stability, hydrophobicity and purity. Based on this screening, five potential candidates were synthesized and tested for minimum inhibitory concentration (MIC) against foodborne pathogens and spoilers. In particular, peptide ORF3663 from *Phaseolus vulgaris* showed promising MIC results against *E. coli* (256 mg/L), *S. Typhimurium* (512 mg/L), *B. thuringiensis* (256 mg/L), *L. innocua* (32 mg/L), *C. sporogenes* (4 mg/L) and
C. tyrobutyricum (1 mg/L). Subsequently, it was tested in cheese and meat models and good inhibition capacity was observed for *L. monocytogenes* and *B. thuringiensis*. The outstanding antimicrobial capacity of this peptide led to cytotoxicity testing to rule out any potential hazards. Our work highlights the benefits of using genome derived data to explore alternatives to antimicrobials with great bacteriostatic and bactericidal activity, conferring sustainable solutions for the race against antimicrobial resistance across the food, clinical, veterinary, and environmental field.

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Developing a co-culture system for producing a bacterial cellulose-hyaluronic acid composite

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In the last years, bacterial cellulose has been intensively studied for the possibility to be easily functionalized by adding materials via ex-situ and in-situ strategy. However, instead of using purified materials, multi- microbial systems, in which microorganisms are cultivated together obtaining combined composites directly during microbial growth are arising as cost-effective strategies. The co-cultivation of bacterial cellulose producers and hyaluronic acid producers would result in a composite with essential features (e.g. moisturization properties) for potential biomedical and cosmetic applications. In this frame, we developed a co-culture system combining two *Komagataeibacter* sp. strains (UMCC 2947 and UMCC 3071) and two *Lacticaseibacillus* sp. strains (UMCC 2496 and UMCC 2535) to obtain a bacterial cellulose-hyaluronic acid(BC-HA) composite. Four different combinations were tested: UMCC 2947- UMCC 2535 (C1), UMCC 2947- UMCC 2496 (C2), UMCC 3071- UMCC 2535 (C3), and UMCC 3071- UMCC 2496 (C4). Changes in BC-HA composites chemical structure and morphologies were investigated by Fourier-transform infrared spectroscopy, scanning electron microscopy, and X-ray diffraction. Water absorption, uptake and antibacterial properties were also tested. Outcomes highlighted the presence of HA in all the composites, among which C3 had the highest content (9.39 mg/g of dried BC). BC-HA composites presented wider fibers compared to pure BC, mainly due to the presence of HA which led to a decrease in composites' crystallinity. Water uptake and water holding capacity showed opposite trends. Indeed, C1 and C2 absorbed less water than C3 and C4, but the water holding capacity was higher. Finally, C1 composite was enriched with a thymol solution to evaluate the antibacterial activity. Composite showed a high antibacterial activity against *Escherichia coli* DSM 30083T and *Staphylococcus aureus* DSM 20231T.

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Wine lees as a substrate for the production of polyhydroxyalkanoates (PHAs)

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Biomass is a renewable, carbon-neutral resource, and it is the perfect candidate for sustainable production of energy, fuels, materials, and chemical products. Italian wine is one of the most important agro-industrial products of the Mediterranean Area. As reported by the International Organization of Vine and Wine, $4.75x10^7$ hectoliters of wine have been produced in Italy in 2020 and, during the winemaking process, an enormous quantity of by-products and waste is generally obtained, with wine lees as the main solid material remaining after alcoholic fermentation. Although several valorization strategies have already been proposed, such as the extraction of antioxidants and the recovery of tartaric acid and ethanol, the production of polyhydroxyalkanoates (PHAs), the so-called "bioplastics", was never explored. The utilization of such residues as carbon sources could also contribute to the reduction of the PHAs production costs, which strongly depends on the substrate availability and price.

The purpose of the present study was to evaluate different bacterial strains, such as *Cupriavidus necator* DSM 545 and *Hydrogenophaga pseudoflava* DSM 1034, for their growth and PHAs accumulation in the liquid phase of Prosecco wine lees. Several wine lees pretreatments (ie, autoclave, centrifugation, filtration and/or pH adjustment) were considered and those supporting the highest bacterial growth were identified. In the ameliorated conditions, *Cupriavidus necator* DSM 545 yielded the highest PHAs content (about 60% of cell dry matter).

This study demonstrates the potential of wine lees as a cheap carbon substrate for the PHAs accumulation by *Cupriavidus necator* DSM 545 and *Hydrogenophaga pseudoflava* DSM 1034. Further studies are ongoing to improve the biopolymer yields by further optimising substrate treatments, inoculum size as well as media composition and up-scaling the process.

These promising results open the way towards effective low-cost PHAs production from wine waste streams.

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Use of plant growth promoting bacteria on Mediterranean crops. Robustness and effects on plants

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A projection of world population of 9.1 billion and an increase of 70 % of global demand for major grain crops within 2050 was reported by FAO (Food and Agriculture Organization of the United Nations). To address this challenge, crop production must increase, with a focus on sustainability avoiding the increase of GHG (Green House Gas) emissions. In this context, the use of Plant-Growth-Promoting Bacteria (PGPB) could be a leading strategy to increase nutrient and water use-efficiency, thus reducing input and gas emission.
PGPB can exert beneficial effects on plant growth, with direct/indirect mechanisms, including P-solubilization capacity, the release of complexing or mineral-dissolving compounds, e.g., organic acid anions, siderophores, protons, and hydroxyl ions, liberation of extracellular enzymes (biochemical P- mineralization), increasing N-availability and phytoprotection.
This research focuses on the use of PGPB, of wild origin (isolated and characterized by the researchers of Predictive Microbiology Lab, University of Foggia) as well as on some commercial bioformulates, used on wheat and on other crops both under controlled conditions (growth chamber) and field trial.
The results suggest the ability of PGPB to modulate soil microbiota, reducing some taxa, as well as to prevail and to survive in soil; in addition, the effects of PGPB on some crop parameters are significant, thus pointing out the practical implications of this approach.

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Microencapsulation and sonication: a multiple physical approach toattenuate the probiotic *Lacticaseibacillus casei* ATCC 393

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Modern consumer demands for sustainable and tasty alternatives to classic dairy products as a vehicle for probiotics. Plant-based beverages meet these requirements but the so-called 'probiotic off-flavour' is a major challenge to consider. It is mainly related to the probiotic acidification abilities that can be counteract by metabolic attenuation. Therefore, the aim of the work is to study microencapsulation and sonication as attenuation strategies on the probiotic *Lacticaseibacillus casei* ATCC 393. Microcapsules with four alginate concentrations and chitosan-coated microcapsules were tested. Microcapsules morphology and entrapment efficacy were also evaluated. Two ultrasound treatments were applied and the probiotic response was also defined in terms of cultivability. Finally, the two technologies were combined. All the samples were added (1 %) to MRS broth, incubated at 37 °C and monitored for pH decrease after 6 and 24 h. The porous structure of the alginate, independently of the concentration, did not prevent bacteria-environment interaction, therefore the acidification occurred. The external layer of chitosan, instead, reduced the porosity, increased the thickness and homogeneity of the microcapsule. Thus, improved its permeability and attenuation capabilities. Moreover, the alginate concentration affected the microcapsule shape that is proved to have a central role in the microcapsule performances. The entrapment efficacy was > 90 %. A strong correlation was found between the intensity of the sonication treatment and attenuation. The 6 min treatment temporary modulated the probiotic acidification abilities, which restore its metabolism after 24 h. Instead, a long- lasting effect was found for the 8 min treatment that has a significant lower pH. Moreover, probiotic cultivability was significantly decreased after ultrasound exposure. Finally, the most efficient system was obtained when the 8 min sonicated probiotic was encapsulated in the chitosan-coated microcapsule (pH 0.31). This study underlines that through a multiparametric approach is possible to efficiently attenuate the metabolism of the probiotic *Lacticaseibacillus casei* ATCC 393.

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Effect of functional ingredients obtained from fermented fish by-product or fish wastewater on safety and shelf-life of amberjack fish balls

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Fish industry already produces a huge amount of wastes and by-products which can represent up to 70 % of the total fish weight. Although most of them are disposed of as waste, these materials are still rich in proteins, lipids and polysaccharides that can be extracted or valorized through technological and biotechnological processes to get functional ingredients applicable in the food industry. Within the framework of the EU project NewTechAqua, and to pursue the goals of a circular economy, two main ingredients, a protein concentrate obtained from fish washing wastewater (PCFW) using pH-shift processingand a fish by-product hydrolysate (FBPH) produced with *Yarrowia lipolytica*, were applied in fish ball formulations made with amberjack minced meat mechanically separated from fish filleting by-products. The aim of the study was to assess the impact of these ingredients on the safety and shelf-life of the new formulated fish balls. In particular, physicochemical parameters (pH and aw), microbial counts (mesophilic bacteria, Enterobacteriaceae, *Escherichia coli*, coagulase-positive Staphylococci, *Pseudomonas* sp., Lactic Acid Bacteria, yeasts, psychrophilic bacteria), safety (presence/absence of *Listeria monocytogenes* and *Salmonella* sp. and biogenic amine content), and volatile molecule profile of the samples were evaluated over time during 20 days of storage at 4 °C in modified atmosphere. While PCFW had a lower impact on the microbiological characteristics of the product, the addition of FBPH improved the acceptability of the new formulation. In fact, total mesophilic bacteria, Enterobacteriaceae, and *E. coli* reached levels above 7, 4 and 2 Log CFU/g, respectively, after 12 days instead of 8 days, as observed in the control. Moreover, addition of FBPH determined a lower accumulation of biogenic amines and release of off flavors. Overall, this approach that combines different strategies can increase the sustainability of the seafood industry by reducing the amount of waste.

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The SUS-MIRRI.IT project: research, services and training for sustainable bioscience and bioeconomy by the MIRRI italian research infrastructure

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SUS-MIRRI.IT (www.sus-mirri.di.unito.it) is a project funded by the National Recovery and Resilience Plan (PNRR), granted by the European Commission's NextGenerationEU programme, with a total budget of about € 17,000,000. The project, coordinated by the University of Turin, involves 15 Institutions with 24 Operative Units (OUs): the Universities of Turin (Coordinator), Cagliari, Genoa, Milano Bicocca, Modena and Reggio Emilia, Naples "Federico II", Palermo, Perugia, Parma, Sassari, Verona and the University of Basilicata, as well as the OGS Institute, CNR (with 7 units: ISA, IPSP located in Turin and Bari, IRSA, ISPA, IBBA, ICB) and ENEA (with 4 units located in Brindisi, Portici, Trisaia and Casaccia). The culture collections of all UOs hold different microbial resources, including bacteria, filamentous fungi, yeasts, microalgae and viruses, which can be exploited in the Health, Agro-Food and Environment domains. Moreover, SUS-MIRRI.IT OUs collect and store data associated with microorganisms, carry on research and provide external users with support and services related to microbial resources. SUS-MIRRI.IT aims to develop research, services and training within the Italian network of culture collections MIRRI-IT. Through the project, the bioresources stored at the MIRRI-IT OUs will be increased and better characterized optimising their management, thus unlocking their genomic and metabolic potentials. The improved management of the resources coupled with the digital platform and data handling/sharing strategy will lead to further discoveries and to the establishment of innovative solutions and products of biotechnological interest, stimulating the bio- and circular economy. The strategic impact of the project will be:

- Promotion of partnerships on the territory to enhance the synergies and favouring the aggregation of skills, structures and bioresources;
- Promotion of the development of the bio-based economy, contributing to the sustainability and safety of the environment and industrial processes;

Targeted initiatives and stakeholder-oriented actions will assure that all results produced by SUS-MIRRI.IT will be disseminated, and, when possible, transferred to industry, promoting the transition to the green economy.

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Antibacterial properties and bioactive compounds of *Opuntia stricta* water extract to be used as natural food preservative

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Opuntia stricta is a member of Cactaceae family and is widespread around the world in arid and semi-aridzones owing to the low water requirements. The fruits represent an underutilized product, which could provide a great source of betalains and phenolic compounds when added in the human diet.

In the present study an ecofriendly extraction procedure, using water as the sole solvent, was performed in order to preserve the peculiar properties of the matrix. The extract obtained was analyzed for its compositionin small bioactive molecules and assessed for its *in vitro* antimicrobial activity against target food pathogens. Compositional analyses (HPLC/DAD and HPLC/ESI-MS) evidenced the presence of pigments possessing betacyanin/betaxanthin structures; numerous flavonoids, particularly flavones and flavanols (luteolin and quercetin derivatives) were also detected and identified.

Moreover, antioxidant activity of *Opuntia stricta* Extract (OS-E) trough DPPH (1, 1diphenyl-2-picrylhydrazyl) assay evidenced 100% scavenging activity.

The OS-E evidenced *in vitro*, through well diffusion method, a wide spectrum of activity against *Bacillus cereus*, *Bacillus subtilis* and *Pseudomonas fluorescens* strains registering inhibition halos ranging from 0.3-0.6 cm of size. The largest inhibition halo, equal to 1.10 ± 0.1 cm, was recorded against *Listeria monocytogenes* strain. *In vitro* results could suggest the potential use of OS-E in processed fish products often subject to contamination by *L. monocytogenes*, in order to control the growth of such hazardous food pathogen and toreduced total microbial growth during refrigerated storage, thus extending shelf life.

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Adrion Master on Circular Economy and Bioeconomy: AMOCEAB

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Starting from the need to foster the thematic link between Circular Economy and BioEconomy, both having food waste, biomass and bio-based products areas of intervention, the project "Adrion Master On Circular Economy and BioEconomy (AMOCEAB)" aims to raise specific competences in these fields. In the project 10 partners from 7 different countries (Italy, Greece, Albania, Bosnia and Herzegovina, Slovenia, Croatia, Serbia) are involved.
The main outputs of AMOCEAB are:
1. the creation of a Transnational Network, which through meetings, roundtables, literature review and a Winter School/BootCamp open to the participation of all partner, jointly define the rules and the technical- scientific Master's contents. The network is configured as a sort of an open lab, through which the participants are able to exchange and disseminate their scientific knowledge, but also to acquire new competences on Circular and Bio-Economy and their theoretical and practical applications.
2. the development of a Strategy and Action Plan to foster Circular and Bio-Economy key principles with the creation of a Master's Programme. This academic course is being configured as a transnational learning path, through which students could spend periods of study from abroad to follow different modules/lessons,under the responsibility of each university. The Master will have a practical cut, training students with innovative activities and compulsory internships, necessary for the creation of new professional figures specialized in Circular and Bio-Economy, to be used as "agents of change" by public and private entities, inthe ADRION area.
Specifically, the main topics that will be developed within AMOCEAB deal with i) Environmental Engineering, ii) Food Biotechnology, iii) Biodiversity, iv) Circular Economy, v) Social impact, vi) Sustainable business models, vii) Digitalization.

Funding
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The use of ultrasound can increase biofilm formation in some functional microorganisms

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Ultrasounds (US) represent a non-thermal approach for food preservation, as they exert a significant antimicrobial activity towards pathogen and spoiling microorganisms. However, depending on the power and the intensity of the treatment, US could exert positive and beneficial effects on several bacteria, including the possibility of increasing adhesion ability of probiotic and/or functional species. Thus, the aim of this research was to study how US could improve biofilm formation of some functional strains, that is *Lactiplantibacillus plantarum* (strains c19 and DSM 1055), *Bifidobacterium animalis* subsp. *lactis* DSM 10140, *B. longum* subsp. *longum* DSM 20219, and *B. longum* subsp. *infantis* DSM 20088. Strains were treated through US by modulating power (10, 30 or 50 % of the net power, 130 W), duration of the treatment (2, 6 or 10 min) and use of pulses (0 or 10 s). After the treatment, a biofilm of the strains was let to form on glass slides and the concentration of sessile cells was analyzed for 16 days. Biofilms formed by untreated microorganisms were used as controls. US significantly increased the concentration of sessile cells of *B. longum* subsp. *infantis*, as well as the stability of biofilm produced by *L. plantarum* DSM1055 after 16 days. The variable mainly involved in this positive effect of US was the duration of the treatment, as biofilm formation and stability were improved only for 2 min-treatments. The results suggest practical implication ofa US pre-treatment for various fields (improvement of adhesion of microorganisms useful in food or in the gut, biomedical and environmental industries), although further investigations are required to elucidate the mode of action.

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A sustainable research for artworks biorestoration: Safebiotech project

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In the field of applied research in the field of bio-restoration, sustainable restoration and conservation practices are becoming increasingly popular both to contain the effects of climate change and to ensure greater operator safety through the use of less hazardous products and greater respect for works of art. The adoption of more sustainable experimental approaches is widely shared in the restoration world. This is reflected among restorers in the consideration that their work practices are potentially harmful to the environment (use and disposal of solvents, chemicals and/or hazardous materials). In this context, an analysis model is proposed that will take into account the impacts expressed in terms of CO_2 emission equivalent (CO_2 eq). resulting from the comparison of data from various available laboratory analytical methods, instrumentation, the use (or non-use) of chemicals (e.g. reagents), resources (e.g. water), the qualitative-quantitative production of waste, reuse and recycling rates, and energy consumption. A methodological procedure that can be applied from research laboratories to work sites and that can therefore find a suitable and appropriate "space" in a concluding paragraph. In this way, the impacts of the research and/or rehabilitation intervention, expressed in terms of CO_2 eq. emissions, can have adequate visibility. To accompany the proposal, a model grid is proposed to facilitate the environmental impacts as described above. As a further example, a comparison and assessment of the environmental impact between an in-person conference and a webinar conference could be carried out; a topic that will not lack for food for thought and moments of discussion.

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Biotechnological valorisation of chickpea flour for the production ofinnovative added value ingredients for food formulation

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World population growth is leading to increased market demand of dietary proteins. The use of plant origin sources could cover the population requirements and reduce environmental impact. Chickpeas, *Cicer arietinum* L., are an interesting protein source for the development of tailored protein enriched ingredients. The application of biotechnological processes on chickpea flour could improve organoleptic, nutritional and functional characteristics, making this matrix suitable for the production of innovative foods. In this context, the aim of this work was to evaluate the characteristics of chickpea flour when subjected to a fermentation process carried out by different species of yeasts, Lactic Acid Bacteria (LAB) and bacilli, in order to select the most promising microbial strains for the production of functional and high nutritional value ingredients. The ability of 18 microbial strains to develop individually in a mixture of chickpea flour and water was evaluated. Microbiological analysis showed the ability of all tested strains to grow in the starting matrix. The strong pH reduction resulted by LAB fermentation prevented the growth of unwanted microorganisms. The metabolism of selected microorganisms affected the volatile molecules profiles of the fermented samples imparting new nuances of flavour. In particular, an increase in acids and alcohols, respectively in samples inoculated with LAB and yeasts, was observed. Moreover, the fermentation processes had a marked effect on the antioxidant capacity of the obtained plant ingredients. The higher antioxidant activity was observed inthe mixtures obtained by LAB fermentation. Finally, some microorganisms showed a marked ability to influence the peptide content of the mixture. From the results obtained, it emerged that each microorganism was able to confer peculiar characteristics to the final product. However, to obtain a chickpea flour-based ingredient to produce value-added foods, it is necessary to consider the functional aspects of the fermented ingredient without neglecting the sensory aspect.

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Identification and disruption of quorum-sensing systems in foodborne *Pseudomonas* and *Campylobacter* species

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Raw milk and meat products are sources of a wide range of foodborne-pathogenic and food spoilage bacteriathat impact food quality and safety if survived in foods at the time of consumption. Campylobacteriosis is one of the most occurrent foodborne disease in the EU, with more than 200,0000 reported human cases in the EU in 2019 according to EFSA. In addition, *Pseudomonas* species are of concern for dairy industry due to their ability to produce heat-stable extracellular enzymes (lipase and protease) that are significantly involved in the spoilage process of dairy products. It is postulated that the expression of virulence-related genes (biofilm formation, proteolytic enzymes and toxins in the case of campylobacteriosis) in these species is regulated by quorum sensing (QS) mechanism. QS is employed by the bacteria for inter- and intra-species communication and initiated by molecules called autoinducers that are produced by the bacterial cells. With emergence of antimicrobial resistant bacteria, it becomes intriguing to investigate other sustainable and safe microbial control. *In vitro* studies suggest that QS inhibition is a surrogate for microbial control.

Probiotication of the food products is now a rising interest for the food industry to increase the product functionality. Furthermore, it is recently proposed that probiotics metabolites are sources of QS inhibitors that reduce gene expression of virulence factors. However, this area of research is still at infancy. This project will present preliminary results about presence of QS systems in isolates of *Campylobacter* species and *Pseudomonas* species (proteolytic and non-proteolytic) from raw milk and meat products. Second, the anti-virulence activity of probiotics against *Pseudomonas* and *Campylobacter* by inhibition of QS mechanism.

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Valorisation of industrial bread waste using enzymatic treatmentand sourdough fermentation

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Nowadays, one of the most important categories of food waste is represented by leavened baked goods, such as bread. Over the last decade, many researchers have attempted to find recycling alternatives. This study aims to recycle surplus bread through the enzymatic hydrolysis and green biotechnology of fermentation, such as sourdough fermentation. "Bread slurries", obtained by mixing bread waste flour and water in a 1:3 w/w ratio, were enzymatically hydrolyzed and fermented for 24 h at 30 °C. Four enzymes, alone and in combination, were used to hydrolyze bread slurries: glucoamylase, amylase fongique, protease and amylase- protease mix followed by fermentation using *Lactiplantibacillus plantarum* and *Saccharomyces cerevisiae* as starters. The enzymatically hydrolysed and fermented bread slurries were evaluated for microbial growth and acidification kinetics, total titratable acidity (TTA), as well as sugars (maltose, glucose and fructose), organic acids (lactic and acetic acids), proteins, peptides and free amino acids (FAA) concentrations. Based on the highest cell density (Log CFU/mL) of *L. plantarum*, acidification and lactic acid production, the protease was shown to be the best performing enzyme. Consequently, the sourdough prepared from protease- treated bread waste fermented with *L. plantarum* and *S. cerevisiae* was subjected to bread making trials with its addition at different percentages, i.e., 10 %, 20 %, 30 % and 50 %, corresponding to 2.5 %, 5 %, 7.5 % and 12.5 % of bread waste in the final product, respectively. All the breads were characterized for pH, TTA, leavening capacity, texture, specific volume, alveolation and colour. In combination with sensory analysis ofthese breads, our results indicated the application of an enzymatically treated sourdough as an ingredient for bread making, thus enabling the recycling of bread waste in a sustainable and low-cost concept of the circular economy.

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